EXPLORATION OF THE GENETIC BASIS OF TARO LEAF BLIGHT RESISTANCE IN BREEDING POPULATIONS

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ABSTRACT

Taro (*Colocasia esculenta*) is a clonally propagated root crop that is a staple of Pacific Islanders. Production of taro is reduced by the Taro Leaf Blight (TLB) disease that is present in most of the taro growing regions of the world. Genetic resistance has been identified in taro populations from Palau, Indonesia and Papua New Guinea. In this study, I explore the genetic architecture of TLB resistance in three breeding populations with the resistant material being incorporated into the existing University of Hawaii taro breeding program. A linkage map based on a F_1 population using single nucleotide polymorphism (SNP) markers was developed to identify quantitative trait loci (QTL) linked with TLB resistance in laboratory assays. Sixteen QTL with major and minor effects were identified on different linkage groups. In addition to the mapping populations, 295 genotypes of taro were evaluated for yield attributes using a Hierarchical Bayesian Model. Yield data were collected for six years from 2013 to 2018, with the breeding cultivar 1025-181 being most promising in terms of yield. The QTL identified in this study can be used in Marker Assisted Selection (MAS) of taro for TLB resistance. The promising genotypes for yield and TLB resistance should be included as parents in future breeding programs.

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CHAPTER 1: THE HISTORY OF TARO (*COLOCASIA ESCULENTA* L.)

Introduction

Taro, *Colocasia esculenta* (L.) Schott., is a tropical root crop widely grown in Asia, Africa, and the Pacific (Rao et al., 2010). It is an herbaceous plant that can grow to a height of 1-2 meters (Onwueme, 1999). Taro belongs to the monocotyledonous family Araceae and subfamily Aroideae (Figure 1-1; Sharma et al., 2016). Only five genera (*Alocasia*, *Colocasia*, *Xanthosoma*, *Cyrtosperma*, and *Amorphophallus*) within this large family are edible (Cabrera et al., 2008). Purseglove (1972) identified at least two botanical varieties of cultivated taro: *Colocasia esculenta* var. *esculenta* and *C. esculenta* var. *antiquorum.* The most notable difference between the two is that *C. esculenta* var. *esculenta* has one large corm surrounded by few cormels whereas *C. esculenta* var. *antiquorum* has a small central corm with large cormels emerging from it. However, there are many morphological characters that vary between these two types (e.g. corm shape and size, leaf shape, the color of petiole). Based on such variation, a great number of agronomic landraces/ cultivars of taro are grown worldwide (Onwueme, 1999).

Botanical Description

Taro is a perennial but is mostly grown as an annual. Leaves are entire, glabrous and thick. The leaf lamina is 20 to 50 cm long, oblong-ovate, with rounded basal lobes. The petiole of the plant is 0.5 to 2 meters long with leaves attached to the petiole from the underside (peltate leaves). A notable exception to this is the 'piko' group found in Hawaii that has leaves with basal lobes spread away from the base.

Taro petioles are spongy and have many air spaces internally which could facilitate the aquatic adaptation of taro (Onwueme, 1999). The taro plant has a central corm which is a modified

underground stem and is the major storage organ of the plant. Cormels arise from the main corm and the number varies greatly between cultivars of taro. Some taro genotypes have structures (i.e., rhizomes) that develop laterally along the surface of the soil to some distance away from the mother corms; such structures could be used for vegetative propagation. Taro roots developing from corms or cormels are adventitious and fibrous and restricted to the upper levels of soil.

Inflorescences of taro arise from the leaf axils and are 2 to 5 in number. Each inflorescence has a short peduncle, a spadix, and spathe (Onwueme, 1999; Lebot, 2000) (Figure 1-2). The spadix is enclosed by the spathe. Each spadix has small sessile flowers attached to it. The lower portion of spadix has female flowers; the top portion has male flowers and in between is a zone of sterile flowers. The extreme tip of the spadix has no flowers and is called the sterile appendage. Female flowers consist of a few obovoid or ellipsoid ovaries 0.5 to 1.5 mm in diameter with sessile stigma. Male flowers consist of 2 to 6 sessile anthers fused to form obconical synandrium (Onwueme, 1999; Whitney et al., 1939).

Taro Genetics

Taro is a highly heterozygous, clonally propagated crop with a base chromosome number of 14, and commonly found with different ploidy levels in nature, specifically diploid (2n=2x=28) and triploid (2n=3x=42) (Mace and Godwin, 2002). It was first domesticated in Southeast Asia and spread to other parts of the world. This geographic center of domestication was arrived at from several sources of data, for example, the greatest chromosomal diversity occurs in India, where a chromosome series (2n=14, 28, 42) occurs. In addition, genetic diversity is higher in Asia than in the Pacific (Lebot and Aradhya 1991; Traore et al., 2016; Helmkampf et al., 2017).

Uses

Many parts of the taro plant are edible including corms, leaf blades, petioles and flowers. The taro corm is rich in carbohydrate and has low protein and fat (Gopalan et al., 1977; Temesgen et al., 2015). Langworthy and Deuel (1922) discovered that raw taro starch was completely digested by humans. Corms are also a good source of potassium and vitamins (National Nutrient Database for Standard Reference Release 28, 2015, USDA). Taro leaves can be cooked and eaten as a vegetable and can be a good source of vitamins and minerals such as protein, vitamin A, riboflavin, vitamin C as well as a large amount of dietary fiber (Bradbury and Holloway, 1998; Temesgen et al., 2015).

History of Hawaiian Taro

In Hawaii, taro was brought by the original Polynesian settlers along with other canoe crops, most likely in multiple arrivals (Cho and Yamakawa, 2007; Helmpkampf et al., 2017). Historically, growing taro was not only a means of food production for Hawaiians, but it was deeply bound to their culture and beliefs of world creation. Taro was a primary staple crop in pre-contact Hawaii, and remains very popular in Hawaiian cuisine. The most popular dish is poi which is produced by mashing the cooked corm to a state of a viscous fluid. One of the most popular cultivars for making poi is ‘Maui Lehua’ which has a purple-colored corm. Another common way of eating corms is simply steaming or boiling them and eating as table taro. ‘Laulau’ is a very popular traditional food made by wrapping fish, pork or chicken with taro leaves before cooking. Taro is reported also to have medicinal importance and it was popular in ancient Hawaiian remedies. (White, 1994).

Taro production

Worldwide taro production

While taro is widely cultivated in Asia, Africa, Pacific and the Caribbean (Rao et al., 2010), Nigeria and China are the leading producers of taro in the world (FAOSTAT, 2014) (Figure 1-3). According to the PLANTS Database of United States Department of Agriculture (2016), taro in the United States is produced mainly in Hawaii, Guam, Puerto Rico, American Samoa, Federated States of Micronesia, Marshall Islands, Northern Mariana Islands, and Palau

Taro production in Hawaii

Despite its cultural importance, there has been a constant decline in taro production in Hawaii over the past 15 years (Figure 1-4). In 1900, taro was cultivated on approximately 1200 acres; however, it is currently grown on approximately 400 acres (Cho and Yamakawa, 2007). Total production of taro in Hawaii in 2017 was 1671.94 tons valued at $2,580,000 (National Agricultural Statistics Service, USDA, accessed on 4/29/2018). Taro production in Hawaii, as well as the Pacific and other countries of the world, is constrained by problems such as 1) replacement by other crops; 2) biotic stresses especially diseases that reduce corm yield; and 3) abiotic stresses due to drought and soil nutrient imbalance.

Taro Leaf Blight (TLB) – The most important biotic stress for Taro

Taro Leaf Blight is caused by the fungus-like oomycete *Phytophthora colocasiae* infecting leaves, petioles, and corms (Miyasaka et al., 2013). The first sign of foliar disease is the presence of water-soaked lesions. Initial symptoms occur as small dark brown flecks or light brown spots on the upper surface of the leaves (Ooka, 1990). These spots enlarge to form a circular, zonate form and the color changes to purplish brown to brown. The lesions dry out during the day and new lesion growth develops at night which gives a distinct concentric pattern of lesion development in the leaf. When the spots increase in size, the whole leaf may be destroyed. During the period of high humidity, a whitish ring of sporangia is also visible in the infected leaf (Brooks,

2005). Another symptom of the disease is the presence of yellow exudates from the infection site which dries and form hard deposits (Singh et al. 2012).

Taro Leaf Blight can reduce corm yield by up to 50% and leaf yield by 95% in susceptible cultivars (Singh et al., 2012). When TLB occurs as an epidemic, leaves are destroyed in 10 days or less, whereas under normal conditions, these leaves could survive for 30-40 days. This destruction of leaves will decrease photosynthesis and reduce corm yield (Nelson et al., 2011). One major impact of this disease in Hawaii was the loss of many TLB-susceptible, traditional landraces. Very few Hawaiian landraces survived the impact of this disease, as well as changes in cultivation practices. Miyasaka et al. (2012) found that all existing Hawaiian landraces were susceptible to TLB.

Taro Breeding

Flowering in taro depends on the genotype and the environment (Lebot et al., 2000). In Hawaii, flowering occurs from May to August. In Hawaii and rest of the Polynesia, there are no specialized insect pollinators for pollination and fertilization to occur in the absence of human intervention (Lebot et al., 2000). Hybrids are developed by hand pollinating taro flowers. Cross-pollination is useful as taro is highly heterozygous and clonally propagated which allows selection in the first generation. Although taro is self-incompatible due to the protogynous nature of the flowering structure, self-pollination within a landrace could occur between flowering structures. Pollen is shed by male flowers in the spathe when mature. Female flowers release an aromatic substance which attracts non-specialized insect pollinators in Hawaii. Usually, the spathe opens slightly when female flowers are receptive. Hand pollination is conducted by spreading pollen over the female flowers of an emasculated plant (Figure 1-5). A successful fertilization will produce seeds within a month. Seeds are produced in the fruit which is a berry. A berry can have more than

50 seeds (Lebot et al., 2000). Depending on the number of berries, the number of seeds could be anywhere between hundreds to thousands, the highest being 22,133 in Papua New Guinea (Lebot et al., 2000).

Breeding of taro could be targeted for high yield, taste quality, resistance against diseases, tolerance to pests, earlier maturity, tolerance to dense planting, tolerance to salinity, the incidence of flowering, floral productivity, and/or ornamental traits (Lebot et al., 2000). In Hawaii, taro breeding is focused on incorporating TLB resistance into Hawaiian cultivars. Taro Leaf Blight resistant plants were introduced from all over the world, especially from Palau. Breeding and selection are done for disease resistance with other desirable traits such as palatability of poi (mashed corm) and lack of rhizomes (runners). Trujillo et al. (2002) were successful in developing a cultivar Pa'Lehua that was found to be highly resistant to TLB, had a high yielding capacity, short maturation, and similar taste to the favorite commercial Hawaiian cultivar Maui Lehua. However, it was susceptible to other diseases, its poi did not ferment properly, and produced an odd flavor when corms were infected with *Pythium* rot (Cho et al., 2007).

Rationale for thesis research

Current breeding efforts, under Dr. Susan Miyasaka, are focused on developing Taro Leaf Blight (TLB) resistant cultivars that maintain all the desirable properties of commercial cultivars, such as Maui Lehua. A promising cross was developed between parents '230' and '255', resulting in progeny '1025', many of which were found to be resistant to TLB and are being evaluated for other agronomic qualities. Parent 230 is a cross between Moi (a Hawaiian landrace) and P-20 (a Palauan landrace). In the same way, Parent 255 is a cross between an F_1 genotype (Red-Moi×PH-15) and Sawahn Kurasae (a TLB-resistant landrace from Indonesia). Genotyping by sequencing

(GBS) has been conducted on 94 individuals from the '1025' cross, along with the two parents, exploring 2447 single nucleotide polymorphism (SNPs) (Helmkampf et al., 2017).

Quantitative traits have a continuous distribution of values because they are controlled by many genes that have effects on phenotypic variation that range from very low to high. Identification and use of molecular markers could help to reduce the number of generations needed to improve cultivars for quantitative traits such as yield, or disease resistance (Bernardo, 2008). Quantitative trait loci (QTL) have been found to be associated with the corm yield and corm dimensions in Vanuatu (Quero-Garcia et al. 2006). Soulard et al. (2017) developed linkage maps using SNP markers obtained from two mapping populations. Shintaku et al. (2016) also created a linkage map using SNPs generated by GBS in 96 progenies from a F_1 population. However, they were not able to find any SNPs associated with resistance/ tolerance to TLB.

In addition to increasing disease resistance, conventional breeding of taro is also conducted to improve yield quality. Hundreds of new genotypes are produced from the breeding program. Plants that pass the initial selection in the field are harvested and evaluated for yield and poi quality. A large number of genotypes are included in field trials with very few or no replications at all. Since growing taro in the field requires space and resources, it is very important to select superior individuals and maintain the breeding stock.

The major objectives of my thesis are to 1) explore the linkage relationship between the SNP markers and QTL for resistance to TLB using three breeding populations, and 2) to advance the current breeding program by evaluating yield data from taro cultivars that were grown for multiple years in Hawaii.

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Tables and figures

Figure 1- 1 Relationships of edible species within the family Araceae. This maximum likelihood phylogeny is based on Phytochrome C gene sequences available from NCBI at *https://www.ncbi.nlm.nih.gov/* was calculated using 1000 bootstrap replications in MEGA7 (Kumar et al., 2015). Alisma Plantago-Aquatica is an outgroup.

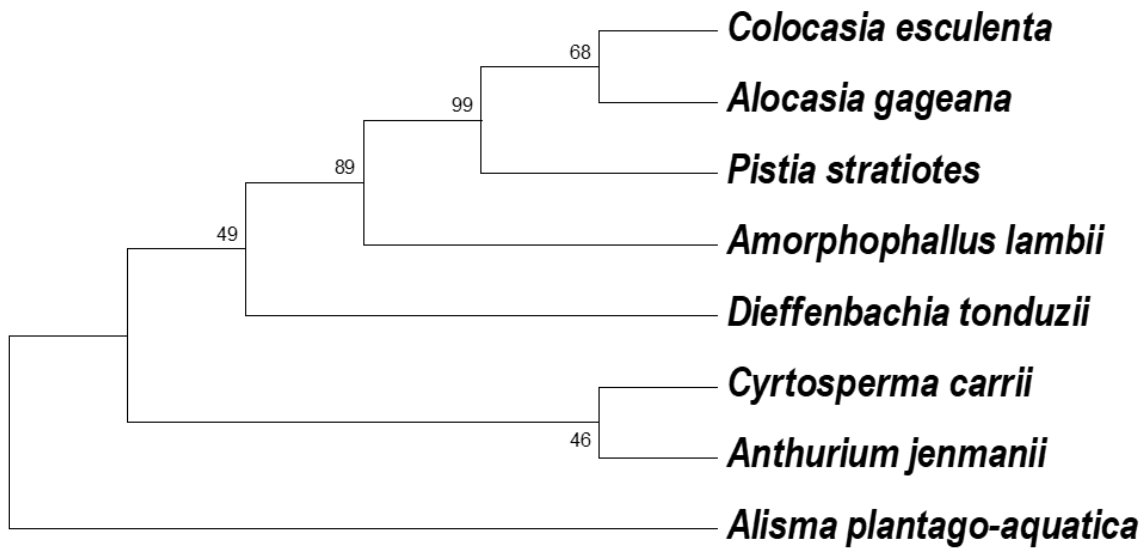


Figure 1- 2 Inflorescence of Colocasia esculenta.

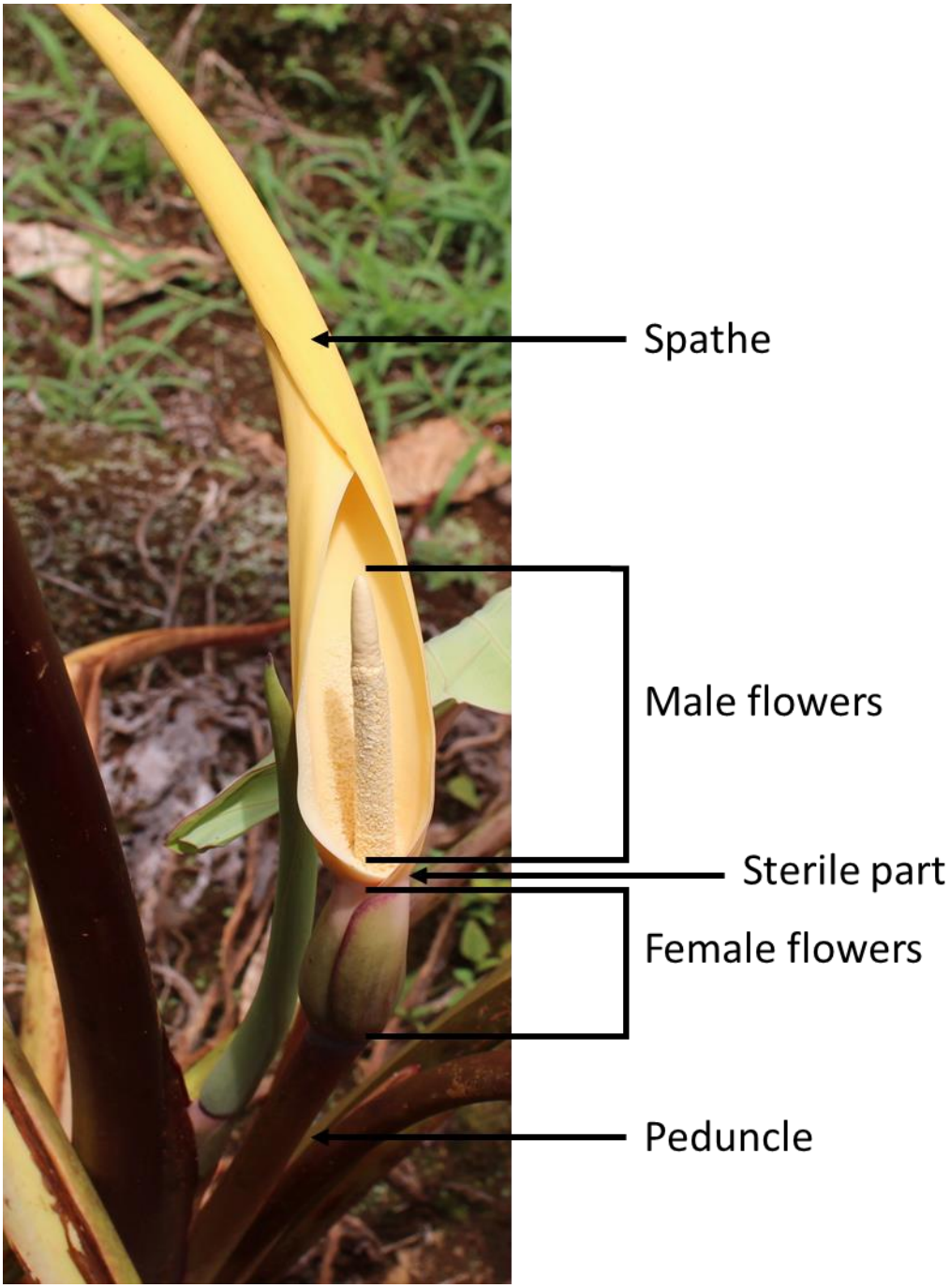


Figure 1- 3 This map is based on the Food and Agriculture Organization (FAO) of the United Nations data and shows average yield over 11 years (2000 to 2014). It is important to note that FAO combined several different species including Xanthosoma spp. into the common name cocoyam for cultivated species phenotypically like taro so these numbers could be an overestimate (Source: FAOSTAT). This map was created with mapchart.net software.

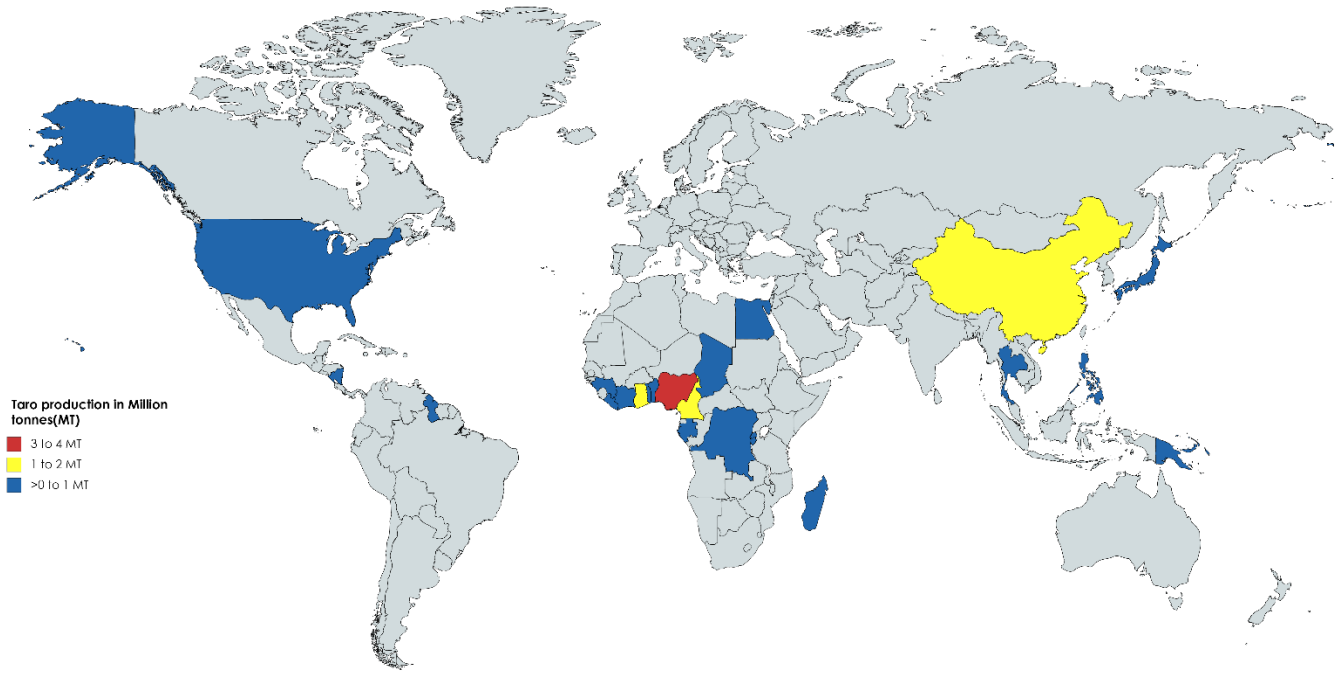


Figure 1- 4 Taro production (mTon) in Hawaii from 1990 to 2015. (Source: National Agricultural Statistics Service, USDA)

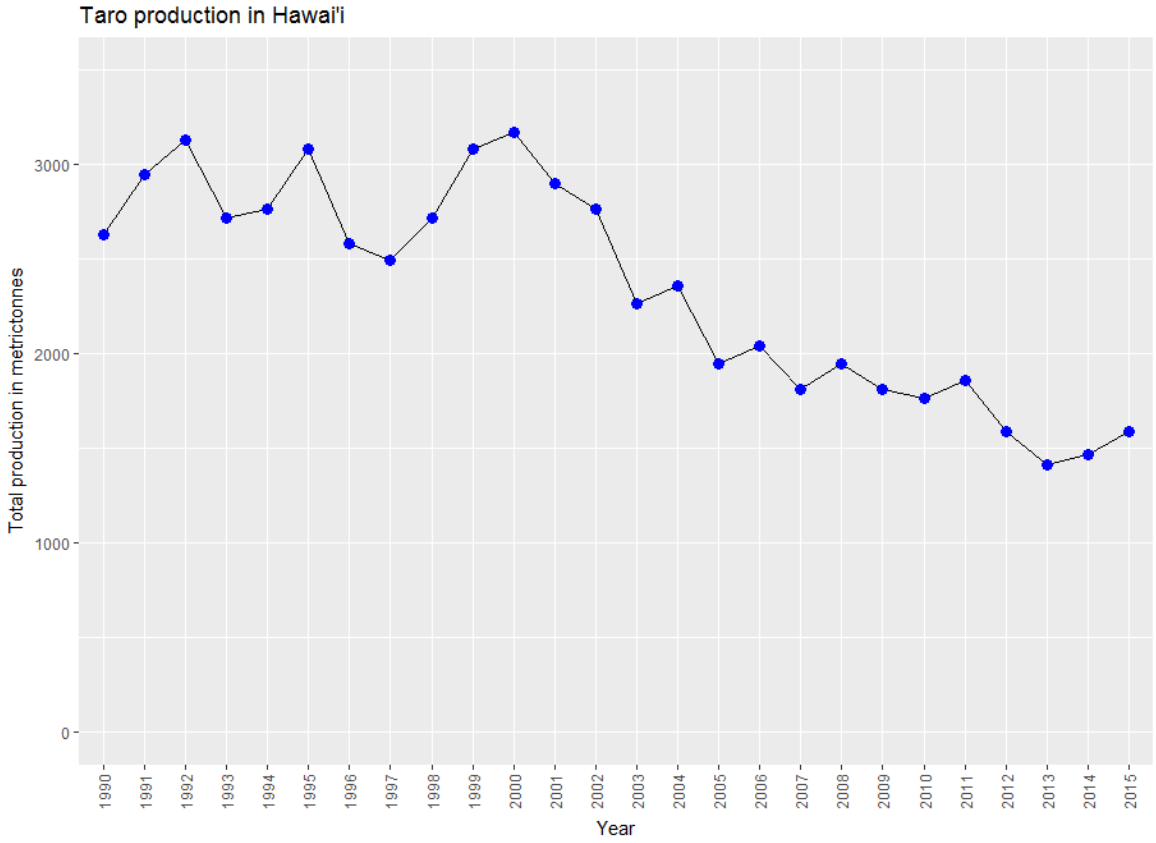
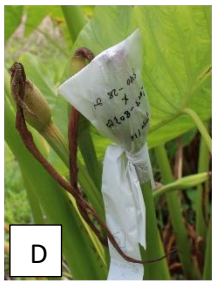


Figure 1- 5 Schematic diagram of the process of taro cross-pollination. A) Find a flower that is shedding pollen B) Emasculate by cutting off the top portion of a flower C) Pollinate the female flower by gently tapping the pollen from the male flower D) Cover the inflorescence with glassine paper bags and tie it with flagging tape. E) Grow seeds in 4-inch pots with germination media.

Pollen collection

Emasculated female plant

Cross Pollination



Bagging and tagging

Three weeks seedlings in nursery

CHAPTER 2: QTL MAPPING FOR TLB TOLERANCE/RESISTANCE

Abstract

Taro Leaf Blight (TLB) is one the most destructive diseases in taro and poses a grave threat to taro production in the world. Owing to the narrow genetic base of taro in Hawaii and much of the Pacific, especially for disease resistance, recent breeding efforts are focused on incorporating resistant materials from outside the local germplasm. The objective of this study is to identify genetic markers linked to TLB resistance. Three mapping populations were used to identify the Quantitative Trait Loci (QTL) linked to TLB disease tolerance. These mapping populations were genotyped using 206 high-quality SNP markers that cover the 14 linkage groups of taro. A detached leaf disk assay was used to phenotype each population. The F_1 population was challenged with isolate S1 and isolate S3, the F_2 population 1063 was challenged with isolate PP8 and isolate UH2 and the F_2 population 1060 was challenged with isolate RP8 and isolate UH2. Inclusive composite interval mapping of Additive and Dominant QTL (ICIM - ADD) method identified 16 QTL across 14 linkage groups in the F_1 mapping population. The QTL identified in this study were found to be specific to be specific to the isolate of *Phytophthora colocasiae*. Twelve QTL were identified for isolate S1 (collected from Pepeekeo, 19.834964°N, 155.100767°W) that explain ~47% of the phenotypic variation for that isolate. The other four QTL for isolate S3 (collected from Pana'ewa, 19.653148°N, 155.049727°W) explain ~52% of the variation in the disease phenotype for that isolate. The highest phenotypic variation by a single QTL was ~19%, confirming the quantitative tolerance observed in the field and a lack of major genes responsible for disease resistance. These putative QTL could be used in future breeding programs to significantly improve the resistance to TLB by expediting the selection process.

Introduction

Taro, (*Colocasia esculenta* L. Schott), ranks fifth among the root crops for production worldwide (FAO, 2014). Taro is a staple food of the Pacific Islanders (Trujillo, 1996), and plays an important role in the food security for the people of South Asia, Sub-Saharan Africa, and the Caribbean (Quero-García et al., 2005; Irwin et al., 1998). Breeding and selection of taro has a long history, with both selection of clonal sports, and hybridization having been practiced throughout history (Lebot, 2000; Helmkampf et al., 2017).

The first modern breeding of taro in the Pacific Islands was initiated in the early 1970s in the Solomon Islands (Lebot, 2000). Breeders at the University of South Pacific were successful in developing a hybrid cultivar "Alafua Sunrise" through sexual hybridization. Alafua Sunrise was superior to existing cultivars in terms of yield, drought tolerance and Dasheen mosaic virus tolerance except for taste (Wilson et al., 1991; Sivan & Liyanage, 1993). Since then, taro breeding has been initiated in many countries of the Pacific. Samoa, Papua New Guinea, and Vanuatu started breeding taro under projects such as TaroGen (Taro Genetic Resources: Conservation and Utilization) and TANSAO (Taro Network for Southeast Asia and Oceania) (Singh et al., 2010).

Scientists of the University of Hawaii-CTAHR started taro evaluations as early as 1936 and made some initial attempts at improving commercial cultivars, but breeding programs have been sporadic (Cho et al., 2007). Hawaiian landraces have many desirable characteristics, such as reddish or purple corm color; resistance to soft rot; low acridity; and early maturity (Cho, 2003). In 1988, Dr. Ramon de la Pena attempted to breed Hawaiian taro cultivars with those from other geographic locations in the collection of taro landraces in Hawaii; however, none of the progeny became commercially successful (Cho et al., 2007). The progenies from crosses between Hawaiian and non-Hawaiian taro genotypes had many undesirable qualities, such as long runners produced

from rhizomes and poor eating qualities. Trujillo et al. (2002) were able to develop hybrids from a cross between Hawaiian cultivar Maui Lehua and TLB resistant Palauan taro 'Ngeruuch'. Three hybrids were selected for release that were highly resistant to TLB and had taste quality similar to 'Maui Lehua'.

In recent years taro breeding has been focused on developing cultivars resistant to or tolerant of Taro Leaf Blight (TLB) (Sharma et al., 2009; Trujillo et al., 2002). Taro Leaf Blight (TLB; *Phytophthora colocasiae*) is a devastating disease that affects leaves adversely by reducing the photosynthetic area and thus severely reducing crop yield. *Phytophthora colocasiae* was first reported in 1900 in Java and now is common in many of the taro growing regions of Asia, Africa and Pacific (Lebot 2000, Raciborski 1900, Trujillo 1967).

When a TLB epidemic occurs there have been large negative impacts on food availability, food prices, and food security in taro growing regions (Singh et al., 2012). The introduction of TLB during the 1920s to Hawaii led to the loss of many Hawaiian landraces that were highly susceptible to this disease (CTAHR, 2009). A recent outbreak of TLB in Samoa in 1990-1993 reduced taro exports from 3.5 million US dollars (58% of Samoa's exports) to 60,000 US dollars (about 1% of Samoa's exports) (Singh et al., 2012).

Taro is a clonally propagated crop in many areas of the world, lacking genetic variation resulting from sexual reproduction. Taro was initially brought to Hawaii by Polynesian settlers and due to the small founding population brought across the Pacific, it has limited genetic diversity compared to south Asian landraces (Lebot et al., 1991; Irwin et al., 1998). Hawaiian taro is very susceptible to TLB, likely due to the absence of disease resistance genes in the Hawaiian taro gene pool (Miyasaka et al., 2012). The temperature and relative humidity of Hawaii is very suitable for growth of the TLB pathogen, providing ample infection potential. Control efforts have included

fungicides; however, this method is expensive and sprays do not work well in taro due to its waxy leaf blade. Further, in countries such as India, the use of fungicides is contributing to the contamination of water and soil system (Sharma et al., 2009).

Breeding of taro is the most sustainable way to cope with fungal diseases. Cho et al. (1997) were able to make a collection of genotypes coming from Hawaii and other parts of the world, and then used a modified backcrossing and recurrent selection strategy where initial F_1's obtained from a commercial Hawaiian cultivar and disease resistant cultivars were backcrossed to Hawaiian parents to create new breeding populations. These populations were again crossed with disease-resistant plants increasing the genetic base for disease resistance (Cho, 2003). These populations were subsequently released for commercial use (Cho, 2003 and Cho, 2007). In a highly segregating population of taro, it is also possible to make selection in the F_1 generation. Trujillo et al. (2002) selected three promising hybrids in the first generation of a cross between Hawaiian cultivar Maui Lehua and Palauan taro 'Ngeruuch'.

Modern genotyping technologies (e.g., Genotyping by Sequencing, GBS) allow for the generation of large numbers of single nucleotide polymorphism (SNP) molecular markers in species with limited genomic resources and for limited cost. The availability of phenotyping and genotyping data facilitates the search for quantitative trait loci (QTL) specific to desirable traits (e.g., disease resistance/ tolerance), and allows for the use of marker-assisted selection increasing the pace of breeding. This study aims to identify the genetic basis for resistance/tolerance to TLB by genotyping three taro mapping populations using SNP markers. These three populations were challenged with two *Phytophthora colocasiae* isolates to better understand the nature of resistance/tolerance to TLB disease in taro and to explore whether multiple sources of resistance

exist. This study had two objectives: 1) Identify the numbers and effects of QTL associated with Taro Leaf Blight tolerance, and 2) Determine if QTL are isolate-specific.

Materials and Methods

Development of Mapping Population and Disease Phenotyping

In a clonally propagated outcrossing species like taro, an F_1 population is typically used for genetic mapping. Here we created both an F_1 and two F_2 populations, specifically, three mapping populations were used for the identification and mapping of QTL associated with TLB resistance: 1) An F_1 population named 1025 based on a cross between breeding lines 230 and 255 (Figure 2-1). Line 230 is a cross between Hawaiian landrace Moi and the Palauan landrace Dirratengadik. Line 255 is a hybrid between the F_1 cross between the Hawaiian landrace Red Moi x TLB resistant Papua New Guinean landrace PH15 and TLB resistant Indonesian landrace Sawahn Kurasae; 2) An F_2 population, labeled 1063, based on self-pollinating the cultivar 2063-803 (Hawaiian landrace Lehua Maoli x TLB resistant Palauan landrace Dirratengadik); and 3) An F_2 population, labeled 1060, based on self-pollinating cultivar 230 (Hawaiian landrace Moi x TLB resistant Palauan landrace Dirratengadik) (Figure 2-1).

The 1025 mapping population was created by Christopher Bernabe (Agricultural Technician, Waiakea Research Station) in the summer of 2012. The F2 population 1063 was also created by C. Bernabe in the summer of 2015 and the F2 population 1060 was developed by Roshan Paudel in the summer of 2016. Briefly, plants with female flowers ready to cross-pollinate were identified and emasculated. Pollen from male flowers was collected and hand pollination conducted. The pollinated flowers for 1025 and 1063 mapping population were tied with flagging tape and the pollinated flowers for 1060 mapping population were covered with glassine paper bags and tagged. Four weeks after pollination, the ripe fruits were collected and processed for

seeds. The seeds were planted in pots with growing medium Sunshine Mix #2 by SunGro Horticulture (Agawam, MA, USA) and kept in a shade house with seedlings being maintained in 4" community pots. The 1025 and 1063 mapping plants were fertilized with Japanese humus Baja Serbajadi (8N:8P:8K:3Mgo) manufactured in Kuala Lumpur, Malaysia. Eight grams (4:3:2) of Nutri-Rich organic fertilizer (Canby, OR, USA) and same amount of slow release fertilizer 'Nutricote' (14:14:14) manufactured by Chisso-Asahi Fertilizer Co., Tokyo, Japan was applied for the 1060 plants and hand irrigation was done on daily basis.

Two strains of *P. colocasiae* were isolated from TLB infected plants at Waimanalo Research Station (RP8) and a taro patch at the University of Hawaii at Manoa (UH2). The S1 and PP8 isolate were collected from taro field at Pepeekeo, Hawaii and the S3 and UH2 (it is different than the isolate from University of Hawaii at Manoa mentioned earlier) isolate was collected from the taro field at Panaewa, Hawaii. Pure *P. colocasiae* cultures were established using a sandwich using a base 10% V8 plates, overlaid with 1.5% water agar plugs cut into a circle ~ 0.5 cm in diameter, which was in turn overlaid with agar plugs from colonized plates previously mentioned (*P. colocasiae* isolation) (Figure 2-2). The two isolated strains of *Phytophthora colocasiae* were used to screen the 1060 mapping population. 'Bun Long' was used as a susceptible check.

The inoculum preparation and virulence assay follow a protocol modified from Brooks (2008). Leaf blade discs with 24 mm diameter were cut from the leaf blades using a cork borer. The second leaf blade from each plant (where leaf blade one is the first fully matured leaf blade) was used in the assay. Leaf blade discs were placed on 0.9% agar with the adaxial surface exposed. Four leaf blade discs from the same genotype were used in separate Petri dishes. *P. colocasiae* cultures were grown on 10% V8 media (500ml: 50 ml V8, .624 g CaCO3, 450 ml water, 10g

agar). The zoospores were obtained between 5 and 10 days. 10 ml water was added to the plate and exposed to 4°C for 30 minutes. The plate is then left for 20 to 25 minutes at room temperature. The plate is swirled gently and the water was pipetted off and place in a tube. Zoospores were counted on hemocytometer. The water is then diluted to approximately 30 zoospores per microliter. Leaf blade discs were then inoculated with 10 µl water containing ~30 P. colocasiae zoospores (Figure 2-3). The plates were covered and left at room temperature and photographed at 48 and 72 hours. Image J software (Schneider et al., 2012) was used to measure the lesion size of the discs.

The lesion diameter of the four discs was averaged and compared with lesion diameter of 'Bun Long'. Relative lesion size was calculated by comparing the averaged lesion diameter of each genotype with the susceptible check genotype Bun Long. A relative lesion size of 0.5 indicates that the individual has half of the lesion diameter of the susceptible check. The F_1 population (1025s) and one of the F_2 population (1063s) were challenged by two isolates of *Phytophthora colocasiae* (isolate S1 and isolate S3 used for 1025 population and isolate PP8 and UH2 for 1063 population). The S1 and PP8 isolate were collected from taro field at Pepeekeo, Hawaii and the S3 and UH2 isolate was collected from the taro field at Panaewa, Hawaii. The phenotyping was done at Dr. Michael Shintaku's laboratory at the University of Hawaii at Hilo. The F_2 population (1060s) was challenged by two isolates of *Phytophthora* (isolate UH2 and isolate RP8). This was done at Dr. Janice Uchida's Mycology lab at University of Hawaii at Manoa.

DNA extraction and genotyping

DNA was extracted from approximately 80 mg of taro leaf tissue using Qiagen DNeasy Plant Mini Kit, following the manufacturer's protocol. The quality of genomic DNA from the 1060 mapping population was checked by gel electrophoresis in a 1% agarose gel. A mixture of 5 µl of genomic DNA and 1 µl of 5X DNA loading buffer was loaded into a well of the 1% agarose gel and let run for 30 min at 80 V. Three µl of the 1 KB hyper ladder (Bioline, Taunton, MA, USA) was loaded on the first well as a fragment size standard. DNA quantification was performed in NanoDrop ND-1000 Spectrophotometer. Approximately 100 µl DNA was placed into separate wells in a 96-well plate and were genotyped by Dr. Kurt Lamour at The University of Tennessee, Knoxville. One plate contained the population 1025 including 93 individuals, one plate contained the population 1060 including 88 individuals, and three plates contained the population 1063 including 276 individuals.

Initial SNP markers were generated from GBS sequencing of 77 taro accessions (63 Hawaiian, 6 South Pacific, 6 Palauan, and 2 mainland Asian) and RAD sequencing of 48 TLB resistant and 48 TLB susceptible progenies including parents 230 and 255 (Helmkampf et al.,2017). These initial 2447 SNP markers generated were filtered for those with appropriate segregation ratios to a group of 787 SNPs which were then used to create a linkage map. This initial linkage map spanned 35 linkage groups (Figure 2-4). A subset of 206 SNPs distributed across the genome from the 787 SNPs were used to design primers to be able to efficiently genotype a large number of individuals. From these 206 SNPs (Figure 2-5), only 187 primers could be developed and reliably genotyped. These 187 SNP sites are thus a subset of markers that passed several rounds of quality control and could be repeatedly amplified in our taro samples.

Linkage mapping

Genetic linkage mapping was conducted using the CDM functionality of GACD software (Zhang et al., 2015) with linkage phases originally unknown. Markers were separated into three segregation types (A=B, AB=CD, and C=D) based on the parental genotypes. An A = B type is where the both alleles at a SNP locus in the female parent are same. If both parents are heterozygous for the two alleles at a SNP locus, an AB=CD code was used. The SNPs that were polymorphic in the male parent were coded as C=D. In case of A=B and C=D markers, loci having BB genotypes or DD genotypes were converted into the heterozygote parental genotype. Markers in linkage groups were defined by positions. The Kosambi mapping function was used to convert the recombination frequency to mapping distance in centimorgans (cM) (Kosambi, 2016). Markers were assigned to linkage groups using a Logarithm of the Odds (LOD) value of 4. A total of 187 individuals from the 1025 mapping population and 206 SNP markers were used for linkage mapping. Genetic mapping of the two F_2 populations was conducted using the R/QTL (Broman et al., 2003) package in Rstudio (R core team, 2015). A LOD score of 3.0 and recombination frequency of 0.4 was used for the 1063 population. Recombination frequencies were converted to centiMorgans (cM) using Kosambi mapping function.

Genotype Imputation

One-hundred and sixty-nine SNP markers were successful in genotyping 94 individuals of the 1025 population, with a portion being monomorphic (uninformative); thus, 28 markers were removed from the analysis. The remaining SNPs were merged to the 206 SNPs developed from the RAD sequencing. MACH 1.0 (Willer et al., 2010) was used for imputing the missing sites. A reference haplotype map was created using 206 SNP markers and 86 individuals. The allele labels in the reference haplotype map and the sample with missing data were compatible and no

inconsistencies in the allele frequencies were found. A Markov Chain iteration was executed 200 times to estimate the missing genotypes. The R-squared value (correlation between imputed and true genotypes) was greater than 0.48 for all markers. An R-squared value of 0.3 was used as a cut-off to avoid poorly imputed markers.

QTL analysis

Phenotypic distribution and normality of the data were checked using statistical software R-3.4.0 (R core team, 2015). Genetic Analysis of Clonal F_1 and Double cross populations (GACD) software (Zhang et al., 2015) was used to identify regions in the taro genome that are correlated with the Taro Leaf Blight resistance in the F_1 population 1025. Inclusive Composite Interval Mapping of Additive and Dominant QTL (ICIM - ADD) was used as mapping algorithm. ICIM is suitable for mapping a small population size as it controls for the bias due to the Beavis effect—i.e., the overestimation of explained phenotypic variance in small populations (Xu, 2003). The LOD threshold was calculated by 1000 permutation tests at $\alpha = 0.05$. Missing phenotypic values were not included in the QTL mapping. Probability of stepwise regression (PIN) value was set at 0.05. The Package R/qtl (Broman, 2003) was used to analyze the marker-trait association for the F_2 populations. QTL mode of action was calculated using the method of Muchero (Muchero et al., 2013).

$$a=\{mu(ac) - mu(bd)\}/2;$$
$$d=\{mu(ad) + mu(bc)\}/2 - \{mu(ac) + mu(bd)\}/2$$

where ‘a’ and ‘d’ are the additive and dominance effects respectively. mu(ac) and mu(bd) are the phenotypic means for the heterozygous loci having alleles from same species. Mu(ad) and mu(bc) are the phenotypic means for the heterozygous loci carrying alleles from both species. The ratio of d/a is used to assess the QTL mode of action, a d/a ratio of <1 indicates underdominance, ratio

between 0 and 1 indicates partial dominance and a ratio of >1 indicates over-dominance (Muchero et al., 2013).

Results

All three mapping populations were challenged with two different isolates of *Phytophthora colocasiae*. Plants within each population showed a quantitative variation in lesion size against different isolates of the pathogen.

F_1 Population-1025s

Phenotype scores

For the S3 isolate, 12% of the plants in the 1025 F_1 population developed no lesions, 74 % of plants had lesions less than the most susceptible check and 14% had lesions size greater than the susceptible check. For the S1 isolate, 50% of the 1025 individuals did not produce any lesions, 23% of the plants had lesion size lower than the susceptible check and 27% of them developed lesions greater than the susceptible check. One individual had the same size as the susceptible check. The distribution of the individuals for the size of the lesion is skewed towards the right—large number of individuals did not develop any lesion or even if they did were lower than the mean value. (Figure 2-6 and Table 1)

Linkage mapping and QTL analysis

A total of 201 markers were assigned to 20 linkage groups (Figure 2-7). Manual ordering within a linkage map was done and loci with suspect linkage were removed from the linkage groups. The linkage map covers a total of 1957.32 cM. The genome wide LOD threshold for QTL mapping was 6.57. If a locus under test has a LOD score higher than the threshold value, its association with the phenotype of interest is statistically significant and thus is declared a QTL.

Composite interval mapping identified 16 QTL on different linkage groups for two isolates of *Phytophthora colocasiae* (Figure 2-8).

Isolate S1

Inclusive composite interval mapping (ICIM) identified 14 QTL with one each on linkage groups 1, 2, 3, 4, 7, 9, 11, and 16, and two on linkage groups 5 and 8 (Figure 2-9A and Table 2). These 14 QTL explained 47.29% of the total phenotypic variation.

Isolate S3

Four QTL were identified for Isolate S3 with one each on linkage group 1, 4, 8 and 13 (Figure 2-9B). Altogether, they explained 52.02% of the variation (PVE) in the trait. The QTL in linkage group 1 was located at 283cM (between scaffold_2727943_129 and scaffold_289773_587) and explained 19.16% of PVE. The QTL in linkage group 8 was identified at 78cM (between scaffold_69776_2168 and scaffold_1336152_191). This QTL explained 14.87% of the phenotypic variation (Table 2).

QTL mode of action

Five QTL (scaffold_24589_2109 and scaffold_161603_938, scaffold_63297_133 and scaffold_755839_271, scaffold_379503_692 and scaffold_68807_501, scaffold_69776_2168 and scaffold_1336152_191, scaffold_3297248_113 and scaffold_47202_1495) had d/a ratio of >1 and thus showed over-dominance, whereas eleven QTL (scaffold_1252928_266 and scaffold_51453_1155, scaffold_363762_540 and scaffold_481335_370, scaffold_379503_692 and scaffold_68807_501, scaffold_36629_930 and scaffold_183564_549, scaffold_114063_1298 and scaffold_280862_865, scaffold_2823_4049 and scaffold_131199_1195,

scaffold_229673_556 and scaffold_23348_66, scaffold_27772_1452 and scaffold_91331_428, scaffold_91539_645 and scaffold_2309241_103, scaffold_2361750_229 and scaffold_16931_2930, scaffold_2727943_129 and scaffold_289773_587) had d/a ratio of <1 which suggests an under-dominance effect.

F2 Population-1063s

Phenotype scores

For the PP8 isolate, 10 % of the 1063 plants developed no lesions, 71 % of plants had lesion size less than that of the most susceptible check and 19 % had lesion size greater than that of the susceptible check. Three individuals had the same lesion size as the check. For the UH2 isolate, 9% of the individuals did not produce any lesions, 73 % of the plants had lesion size lower than the susceptible check and 16 % of them developed lesion sizes greater than that of the susceptible check. Seven individuals had the same lesion size as the susceptible check. The distribution of the individuals for the size of the lesion is skewed towards the right—i.e., tolerant to TLB (Figure 2-10 and Table 3).

F2 population -1060s

Phenotype scores

For the RP8 isolate, none of the plants in the 1060 F_2 population showed absolute resistance. Sixty-six percent of plants had lesions less than the most susceptible check and 28% had lesions size greater than the susceptible check. Five individuals had the same lesion size as the susceptible cultivar. For the UH2 isolate, 92 % of the plants had lesion size lower than the susceptible check and 8 % of them developed lesions greater than the susceptible check (Figure 2-11 and Table 4).

Linkage mapping of the F2 populations

The F_2 mapping population of 1060 and 1063 did not provide useable results for linkage mapping. Of the 206 genotyping assays only 62 markers were polymorphic within the 1063 population and only 77 in the 1060 population. Although linkage maps could be developed, they suffered from poor resolution, and while these maps were broadly consistent with the F_1 map they had very large gaps, resulting in unreliable QTL mapping.

Discussion

The cultivars that were used for cross pollination to develop the mapping population could flower naturally and are amenable to a non-destructive method of phenotyping for *Phytophthora colocasiae*. This method has been found to be quick, easy and reliable for screening taro for TLB disease (Brooks 2008, Nath et al., 2016). However, Shrestha et al., (2017) found the loss of heterozygosity for some SNP markers during the laboratory culture of isolates from Hawaii and hypothesized that it could create a loss of virulence affecting the screening against TLB. The use of single hyphal tip to infect the plants could help to remove inconsistencies in phenotyping (Kurt Lamour, Personal communication). Each population showed phenotypic segregation for TLB resistance.

In this study, the phenotypic data showed a continuous distribution, indicating quantitative resistance. In a similar study done by Shintaku et al. (2016), all of the Hawaiian cultivars used in TLB phenotyping had relative lesion size greater than 0.2. A popular Hawaiian landrace, Red Moi, used to develop our mapping population had a relative lesion size value of 0.72. This parent while popular for poi and table taro, has poor disease resistance. Genotypes that have less than 0.5 relative lesion size are the most promising for resistance/ tolerance selection. In each population

included in this study it was possible to observe individuals with superior disease resistance characteristics that could be selected for future larger scale testing.

Under ideal circumstances, linkage mapping data would be created from genotyping data that has no missing values and every marker would follow Mendelian segregation ratios. However, our data is not ideal, with missing genotypes. Many software packages utilize strategies to handle missing data and genotyping errors; nevertheless, missing data can affect the map order and length (Hackett et al., 2003). The missing marker data can be imputed based on the information present in the neighboring markers as individuals descended from a common ancestor share chromosomal segments with each other. Genotype imputation takes advantage of these shared haplotypes to estimate the markers that are not genotyped (Li et al., 20009). Here, the use of reference haplotype from the same pedigree made the imputation safe and accurate. Although, a few markers still showed segregation distortion after imputation, the overall Mendelian segregation of the remaining markers was improved. The same parameters (LOD = 3.0 and maximum recombination frequency =0.4) that were used for the mapping of 787 SNP markers was used in onemap package (Margarido et al., 2007) in R to estimate the map with the imputed markers. The markers and linkage groups were consistent with the original map (Figure 2-12).

The chromosome number of taro is 2n=2X=28. Linkage mapping using CDM functionality of GACD recovered 20 linkage groups using the 206 SNPs in the 1025 mapping population. It is possible that the small linkage groups in this study (16-20) could be the sub-set of the other 14 linkage groups. The large genome and high heterozygosity has caused difficulty in resolving the expected 14 linkage groups, and similar results were observed also by Soulard et al. (2017). These technical difficulties also have been observed in other clonally propagated tubers such as cassava (Nzuki et al., 2017). Although widely practiced, it is not ideal to create a genetic map using an F_1

population. The number of linked markers can be much less compared with the number of polymorphic markers in an F_1 population (Chen et al., 2010). More recombination in subsequent generation would provide more details of the linkages and ideally would create a linkage group that totally matches the chromosomes.

The markers used in this study were selected based on low segregation distortion and whole genome coverage. In a F_1 mapping population, markers that are homozygous (AA) in one parent but heterozygous (AB) in another parent are informative and they will segregate into 1AA:1AB genotypes. Markers that are heterozygous (AB) in both parents are also informative. The expected segregation ratio in such markers is 1AA:2AB:1BB. However, in a F_2 population only markers heterozygous in the parents are informative, because homozygous markers in the F_2 will not segregate in the offspring. Many of the SNP markers that we used in our F_2 populations were homozygous for the F_1 parent. This limited the number of markers useful in linkage mapping of F_2 population. In the presence of relatively few markers, an accurate linkage map could not be created. Although the map was not perfect, linkage mapping of F_2 population 1063 was better compared to the F_2 population 1060 (Figure 2-13). The majority of markers were assigned to some linkage groups in 1063. In contrast, the presence of few markers coupled with a relatively small population size lead to a poor resolution linkage map with many markers assigned as unlinked in the F_2 1063 mapping population. (Figure 2-14).

Sixteen QTL were identified for two isolates of *Phytophthora colocasiae* in our F_1 population. For isolate S1, 14 QTL were identified, although none explained more than 10% of the phenotypic variance, indicating they were minor effect QTL. For the S3 isolate, four QTL were identified with three explaining more than 10% phenotypic variation. One QTL for isolate S3 that mapped to LG1 at 283cM explained 19.15% of the phenotypic variation. The ICIM method was

used for QTL mapping to identify small effect loci in a small population. This method could be the reason why so many QTL were identified for the S1 isolate which had a relatively larger population of 187 individuals and fewer for the S3 isolate which had a relatively smaller population of 91. Of the 16 putative QTL identified in our study, 15 of them were isolate-specific with only one in common between the isolates. This result is similar to other studies of *Phytophthora* species where isolate-specific effects were found (Ewing et al., 2000). This phenomenon underlines the need for stacking resistant genes, because multiple races and mating types of *Phytophthora* are found in Hawaii (Shrestha et al., 2017).

It is crucial to validate the QTL in different mapping populations before using them for marker assisted selection. Since we have genotypic data on all the SNP markers from the two parents of the 1025 population, a relatively large mapping population could be created from the self-pollination of the line 230 and line 255. Only those SNPs that are heterozygous in each of the parents should be used to assay the F_2 population. In this study, we selected markers based on segregation distortion. However, if we were to genotype a larger F_2 population we would expect the markers to have a better segregation ratio and thus we can use more markers than we used for this study. A larger population and more markers would be lead a more accurate mapping. In the long term, new segregating populations should be created. The line 230 and line 255 could be crossed with Hawaiian cultivars such as Moi and Maui Lehua. Genotyping by Sequencing (GBS) could be done on those populations. High sequencing depth could take care of the segregation distortion to some extent. These multiple populations would allow scanning of QTL over multiple backgrounds. This procedure would provide more insight into linkage mapping by exploring the consistency of linkage maps over different populations.

Conclusions

In this study we explored three populations and 544 individuals. Both major and minor QTL were identified, with 18.56% of individuals showing tolerance to TLB isolates. The sources of resistance were isolate-specific and the sources were from different localities. This study highlights the potential of introducing new germplasm as well as the value of using marker technology to improve breeding efficiency. The selected plants from each population are being advanced for further trials.

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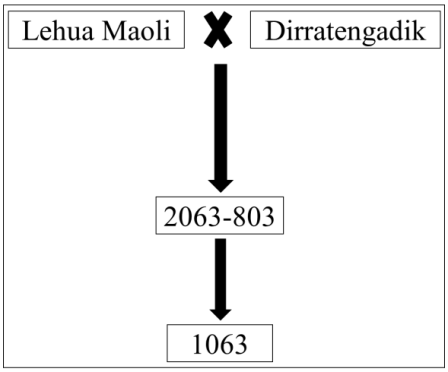
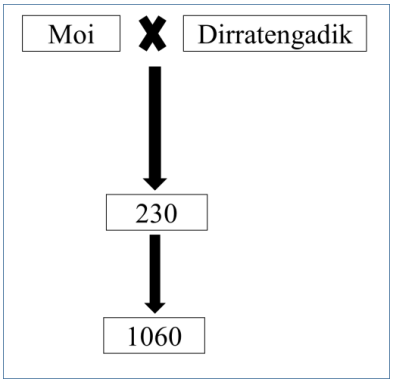
Tables and figures

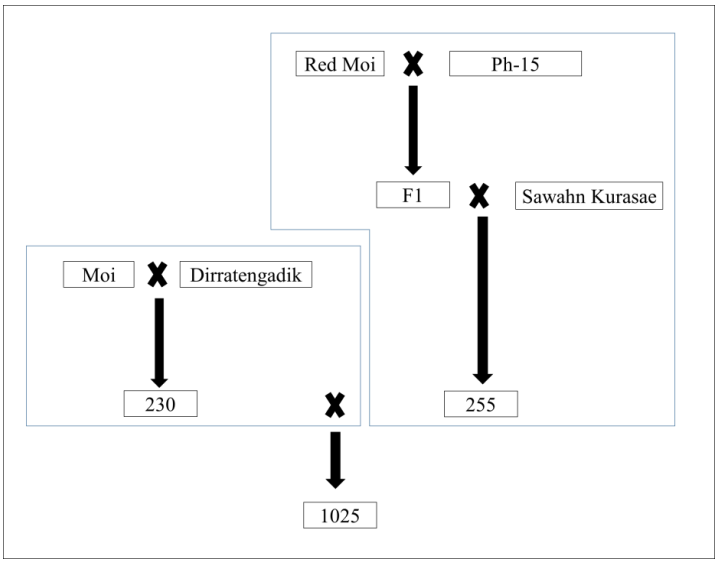
Figure 2- 1 Three mapping populations used for the QTL mapping study: A, F2 population of 1063; B, F2 population of 1060; and C, F1 population of 1025.



A) 1063 mapping population



B) 1060 mapping population



C) 1025 mapping population

Figure 2- 2 Sporangia (as shown by the black arrows) of *Phytophthora colocasiae* isolated from taro cultivar Manini Uliuli at Waimanalo research station identified at 100X. The photograph is taken from water agar isolation plate.

Figure 2- 3 Leaf Disk assay technique. Susceptible Bun Long (BL, upper left on each plate) was used as a check cultivar.

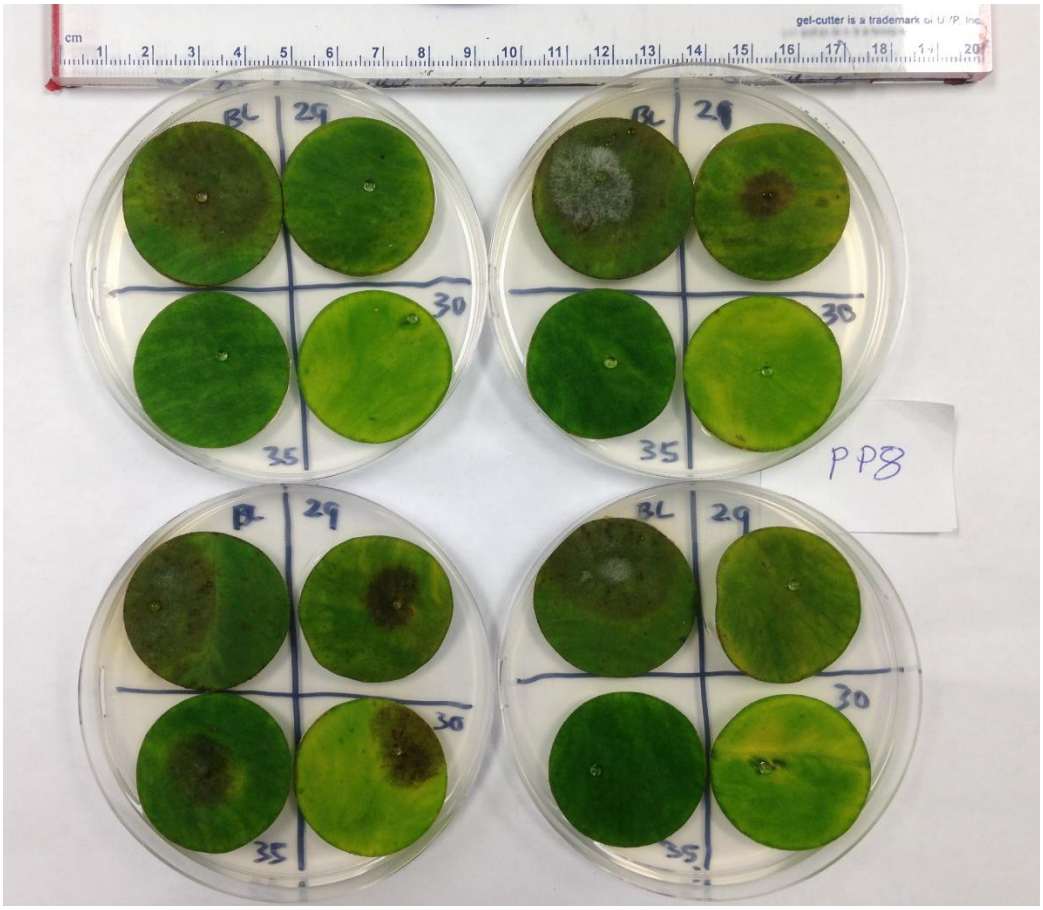


Figure 2- 4 Initial Genetic Linkage map based on 787 SNP markers from RAD sequencing of 96 individuals of the 1025 mapping population (LOD = 3 and maximum recombination frequency = 0.4).

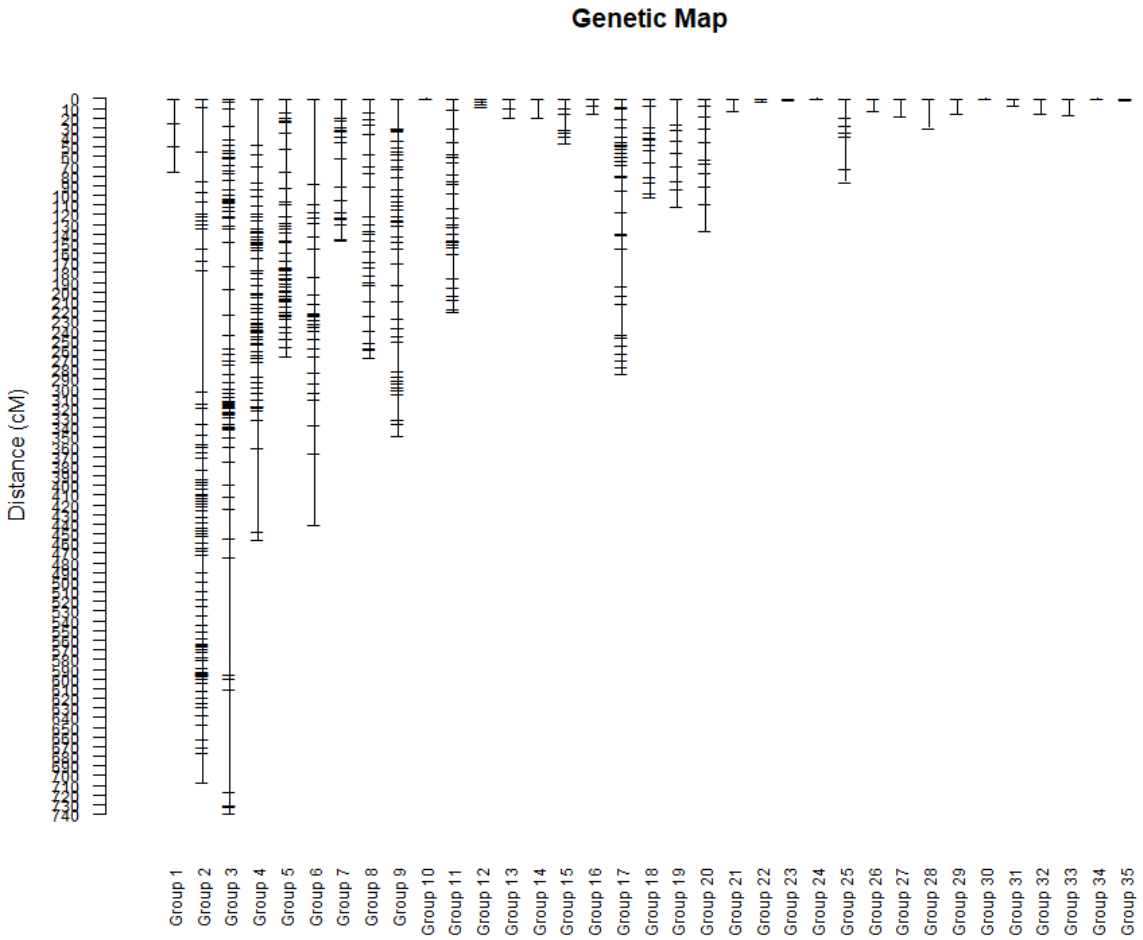


Figure 2- 5 High quality SNPs (206) that were chosen from across 26 linkage groups from the linkage map created using 787 SNPs from RAD sequencing of 96 individuals of the 1025 mapping population.

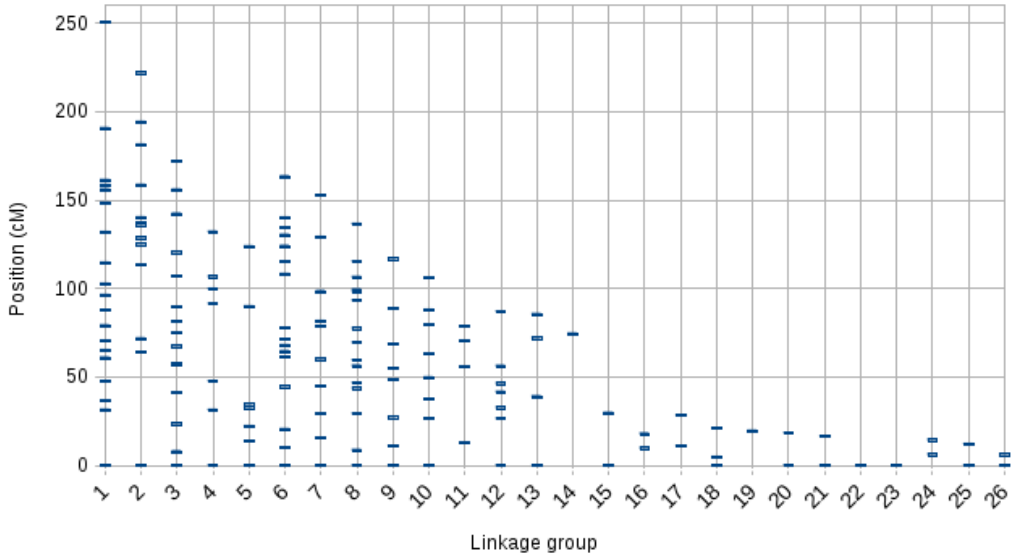


Figure 2- 6 Histogram of Relative lesion diameter for F1 mapping population 1025. Relative lesion diameter is calculated by dividing the average lesion size of individual genotype by the average lesion size of susceptible check cultivar Bun Long. 2-6A) Distribution of Taro Leaf Blight resistance in 1025 population for isolate S1. 2-6B) Distribution of Taro Leaf Blight resistance in 1025 population for isolate S3.

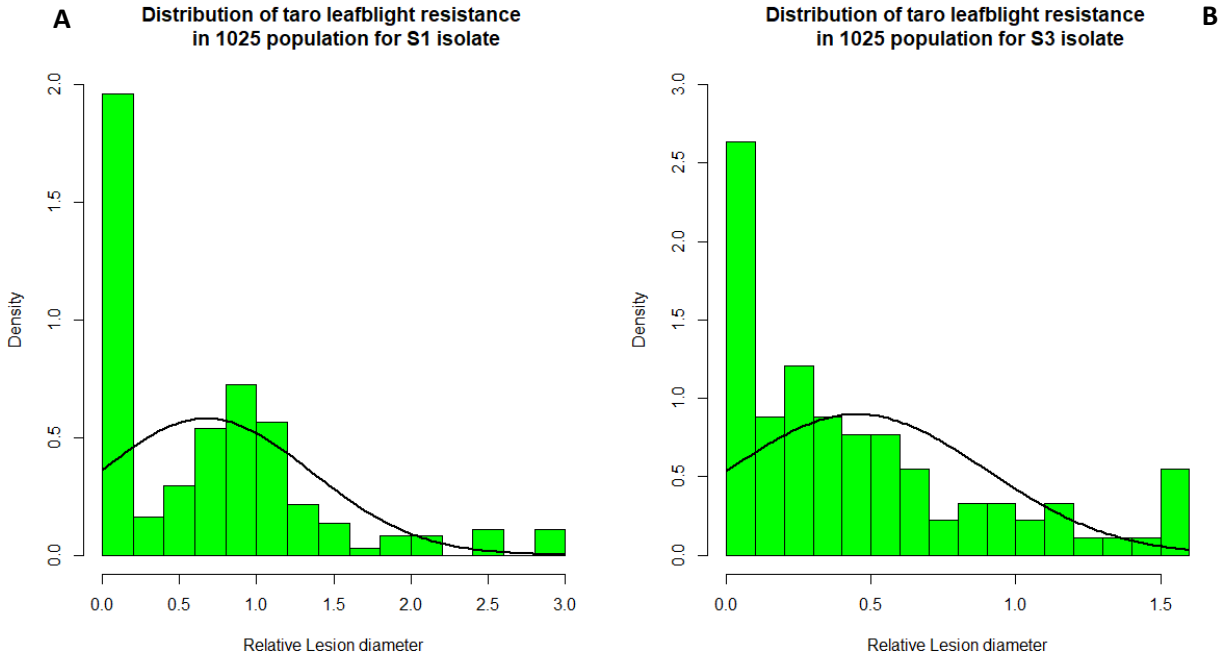
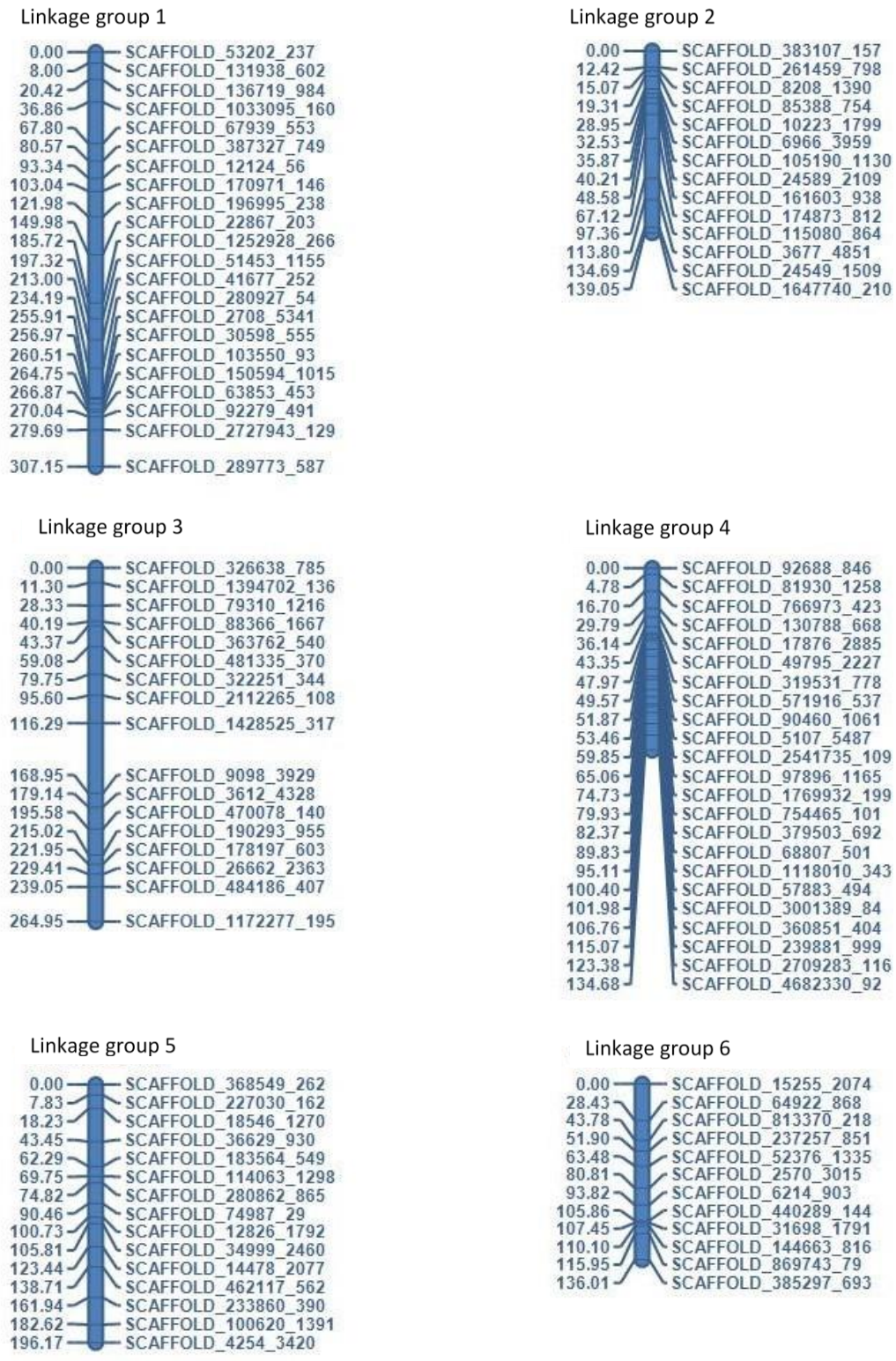
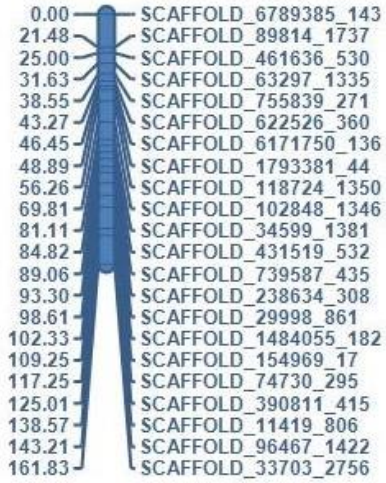


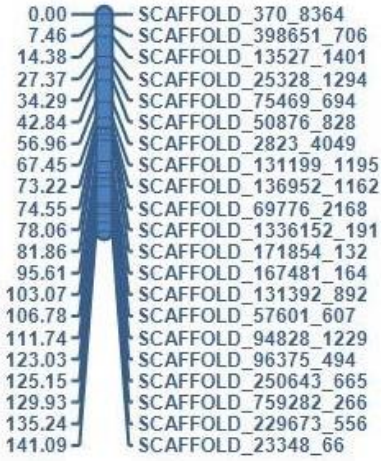
Figure 2- 7 Genetic markers (SNPs) and their position in the different linkage groups in F1 mapping population of 1025.



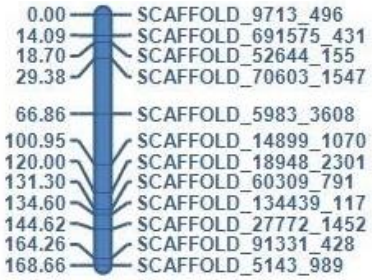
Linkage group 7



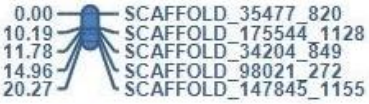
Linkage group 8



Linkage group 9

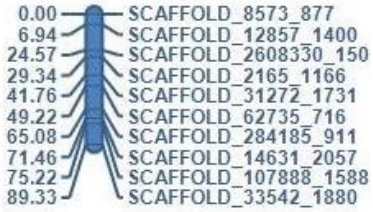


Linkage group 10



Linkage group 11

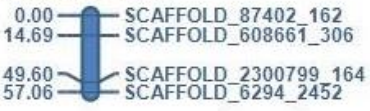
Linkage group 12



Linkage group 13

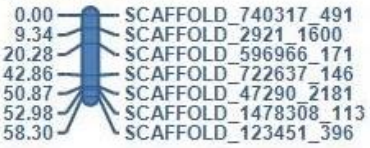
Linkage group 14

Linkage group 15



Linkage group 16

Linkage group 17



Linkage group 18

Linkage group 19

Linkage group 20

Figure 2- 8 Whole genome representation of QTL identified for two isolates of Phytophthora colocasiae in the 1025 mapping population. The Y-axis indicates the logarithm of odds (LOD) values. Peaks above the threshold (dotted line) of LOD=6.57 represent a QTL having significant interaction with the TLB tolerance. 8A) QTL identified for S1 isolate and 8B) QTL identified for S3 isolate.

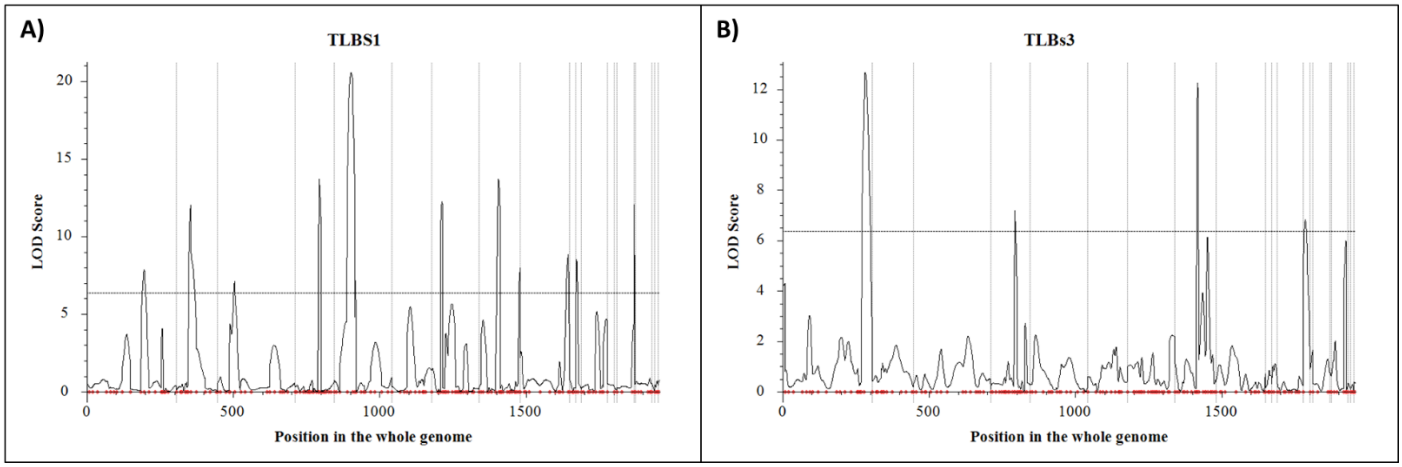
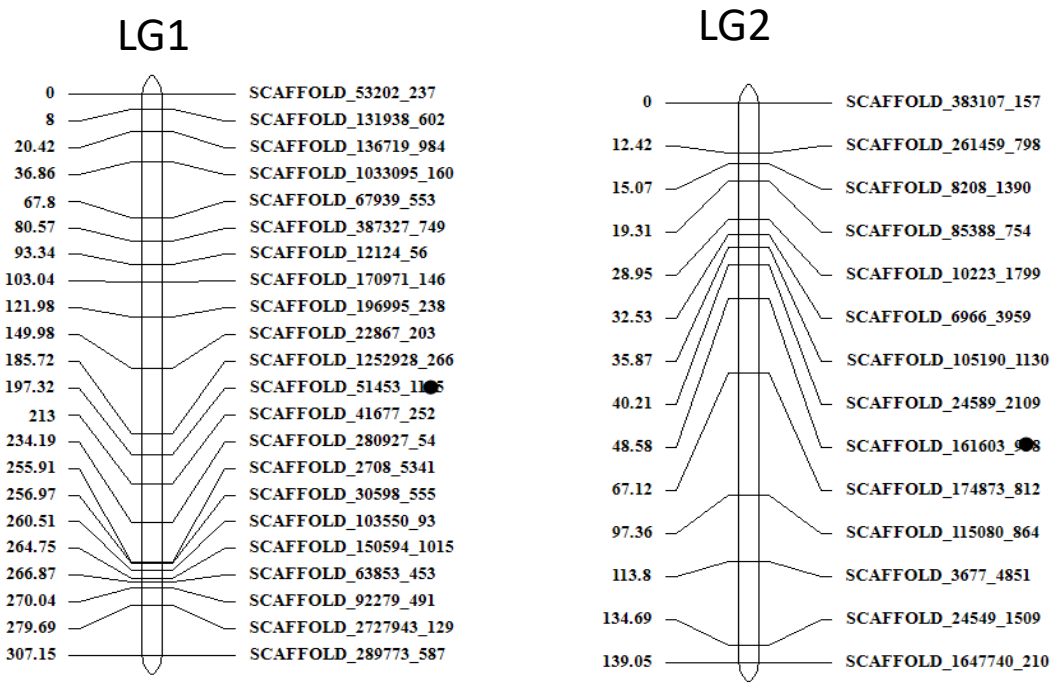
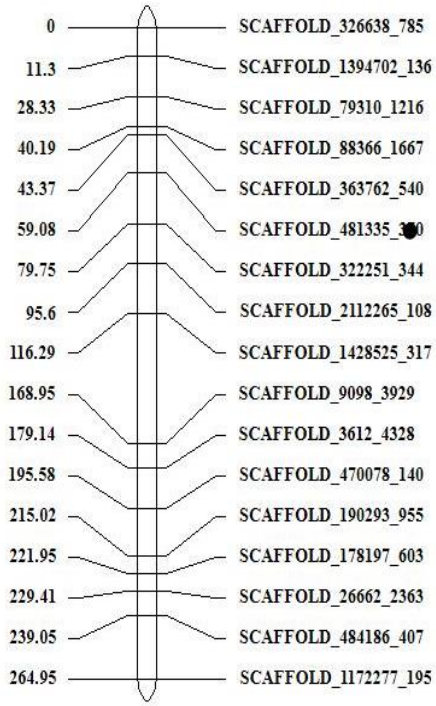


Figure 2- 9 Presence of QTL as shown by the black dots and hills on different linkage groups of 1025 mapping population. 2-9A) QTLs for isolate S1 shown by black dots. 2-9B) QTLs for isolate S3 shown by black hills.

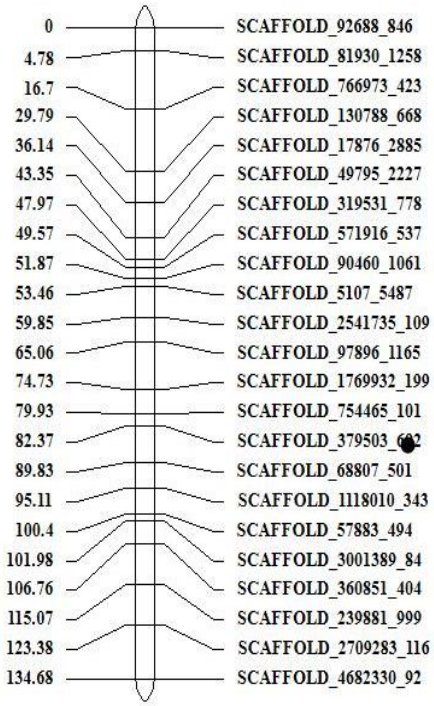
A



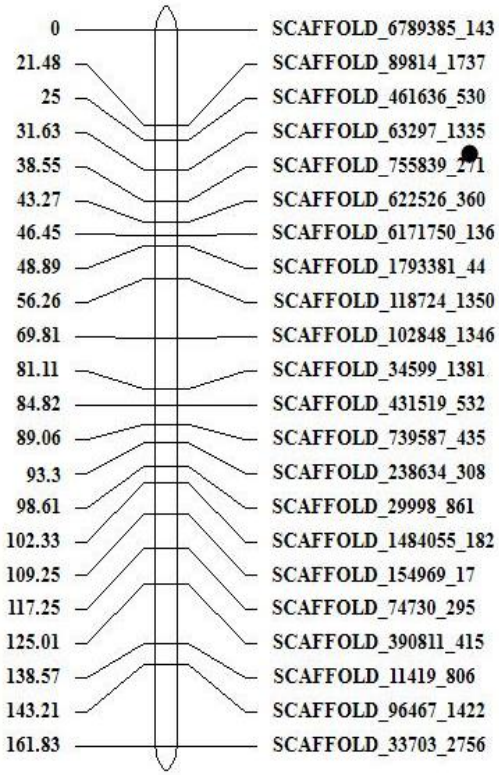
LG3



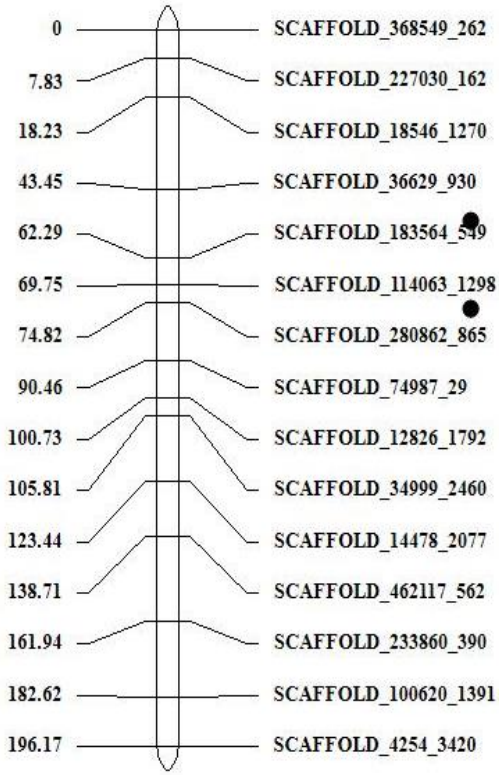
LG4



LG7



LG5



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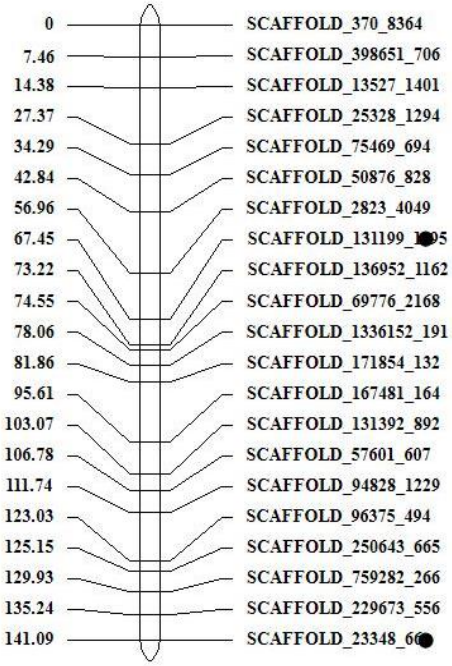
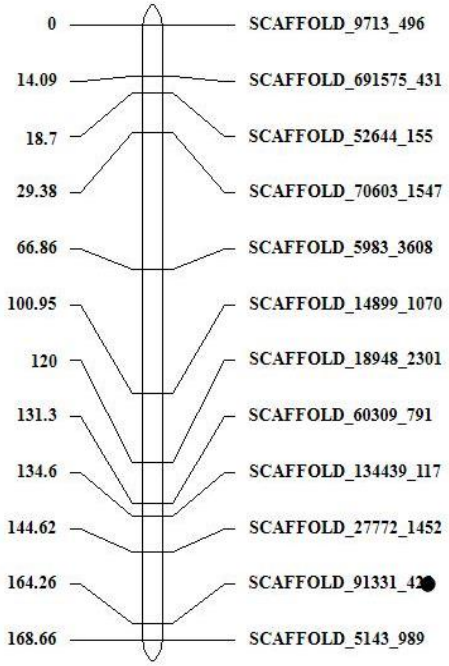
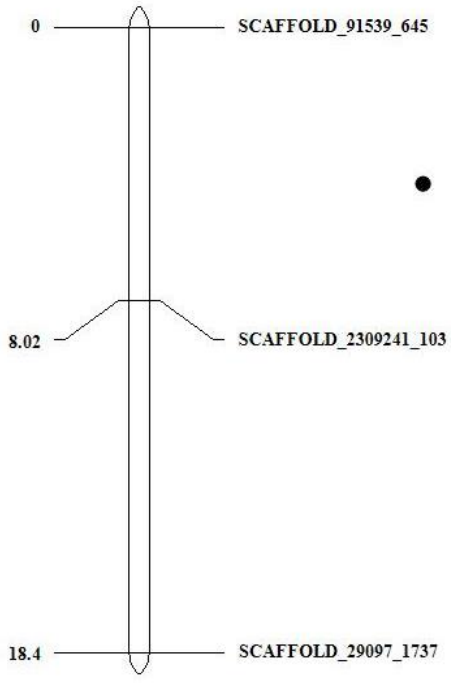


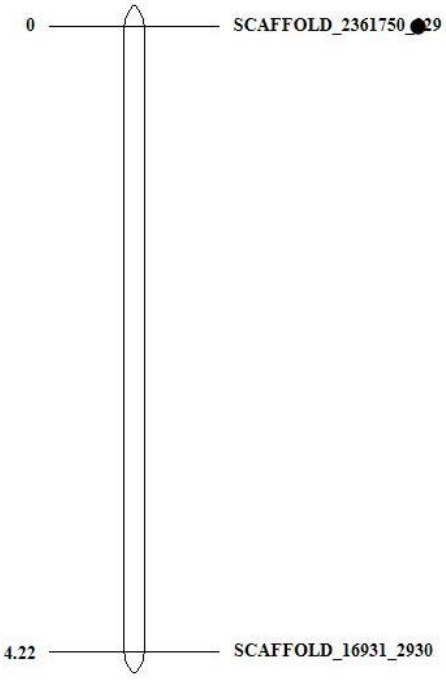
LG9



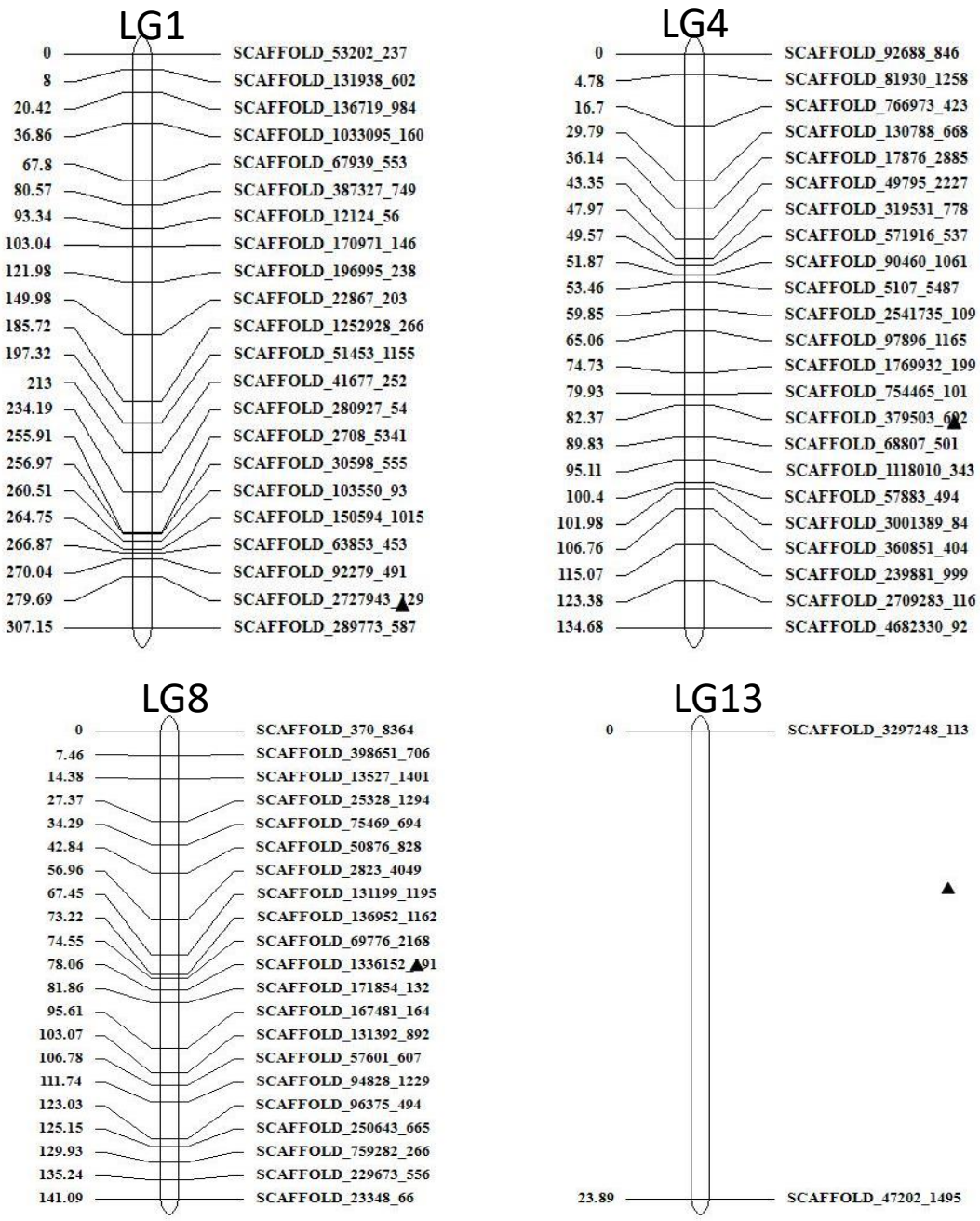
LG11



LG16



B


LG1
0 SCAFFOLD_53202_237
8 SCAFFOLD_131938_602
20.42 SCAFFOLD_136719_984
36.86 SCAFFOLD_1033095_160
67.8 SCAFFOLD_67939_553
80.57 SCAFFOLD_387327_749
93.34 SCAFFOLD_12124_56
103.04 SCAFFOLD_170971_146
121.98 SCAFFOLD_196995_238
149.98 SCAFFOLD_22867_203
185.72 SCAFFOLD_1252928_266
197.32 SCAFFOLD_51453_1155
213 SCAFFOLD_41677_252
234.19 SCAFFOLD_280927_54
255.91 SCAFFOLD_2708_5341
256.97 SCAFFOLD_30598_555
260.51 SCAFFOLD_103550_93
264.75 SCAFFOLD_150594_1015
266.87 SCAFFOLD_63853_453
270.04 SCAFFOLD_92279_491
279.69 SCAFFOLD_2727943_129
307.15 SCAFFOLD_289773_587
LG4
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4.78 SCAFFOLD_81930_1258
16.7 SCAFFOLD_766973_423
29.79 SCAFFOLD_130788_668
36.14 SCAFFOLD_17876_2885
43.35 SCAFFOLD_49795_2227
47.97 SCAFFOLD_319531_778
49.57 SCAFFOLD_571916_537
51.87 SCAFFOLD_90460_1061
53.46 SCAFFOLD_5107_5487
59.85 SCAFFOLD_2541735_109
65.06 SCAFFOLD_97896_1165
74.73 SCAFFOLD_1769932_199
79.93 SCAFFOLD_754465_101
82.37 SCAFFOLD_379503_692
89.83 SCAFFOLD_68807_501
95.11 SCAFFOLD_1118010_343
100.4 SCAFFOLD_57883_494
101.98 SCAFFOLD_3001389_84
106.76 SCAFFOLD_360851_404
115.07 SCAFFOLD_239881_999
123.38 SCAFFOLD_2709283_116
134.68 SCAFFOLD_4682330_92
LG8
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7.46 SCAFFOLD_398651_706
14.38 SCAFFOLD_13527_1401
27.37 SCAFFOLD_25328_1294
34.29 SCAFFOLD_75469_694
42.84 SCAFFOLD_50876_828
56.96 SCAFFOLD_2823_4049
67.45 SCAFFOLD_131199_1195
73.22 SCAFFOLD_136952_1162
74.55 SCAFFOLD_69776_2168
78.06 SCAFFOLD_1336152_491
81.86 SCAFFOLD_171854_132
95.61 SCAFFOLD_167481_164
103.07 SCAFFOLD_131392_892
106.78 SCAFFOLD_57601_607
111.74 SCAFFOLD_94828_1229
123.03 SCAFFOLD_96375_494
125.15 SCAFFOLD_250643_665
129.93 SCAFFOLD_759282_266
135.24 SCAFFOLD_229673_556
141.09 SCAFFOLD_23348_66
LG13
0 SCAFFOLD_3297248_113
23.89 SCAFFOLD_47202_1495

Figure 2- 10 Histogram of Relative lesion diameter for F2 mapping population 1063. 2-10A) Distribution of Taro Leaf Blight resistance in 1063 population for isolate PP8. 2-11B) Distribution of Taro Leaf Blight resistance in 1063 population for isolate UH2.

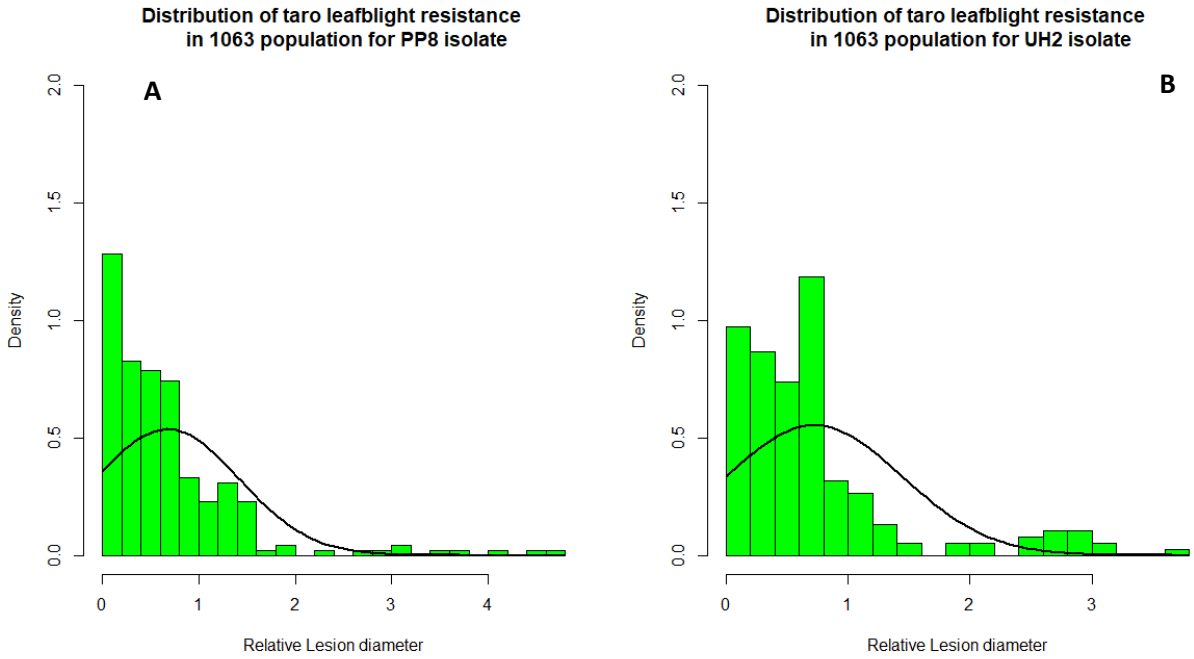

Figure 2- 11 Histogram of Relative lesion diameter for F2 population 1060. 2-11A) Distribution of Taro Leaf Blight resistance in 1060 population for isolate RP8. 2-11B) Distribution of Taro Leaf Blight resistance in 1060 population for isolate UH2.

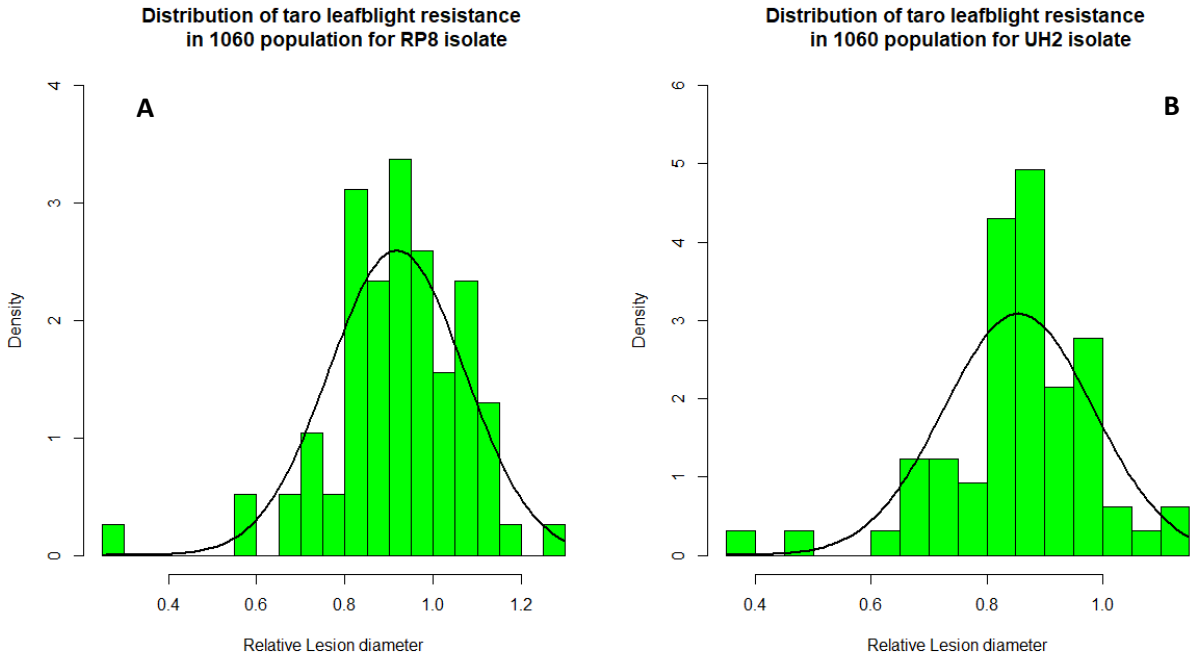

Figure 2- 12 Genetic linkage map with 201 SNPs on 187 individuals of 1025 mapping population (LOD = 3, maximum recombination frequency =0.4).

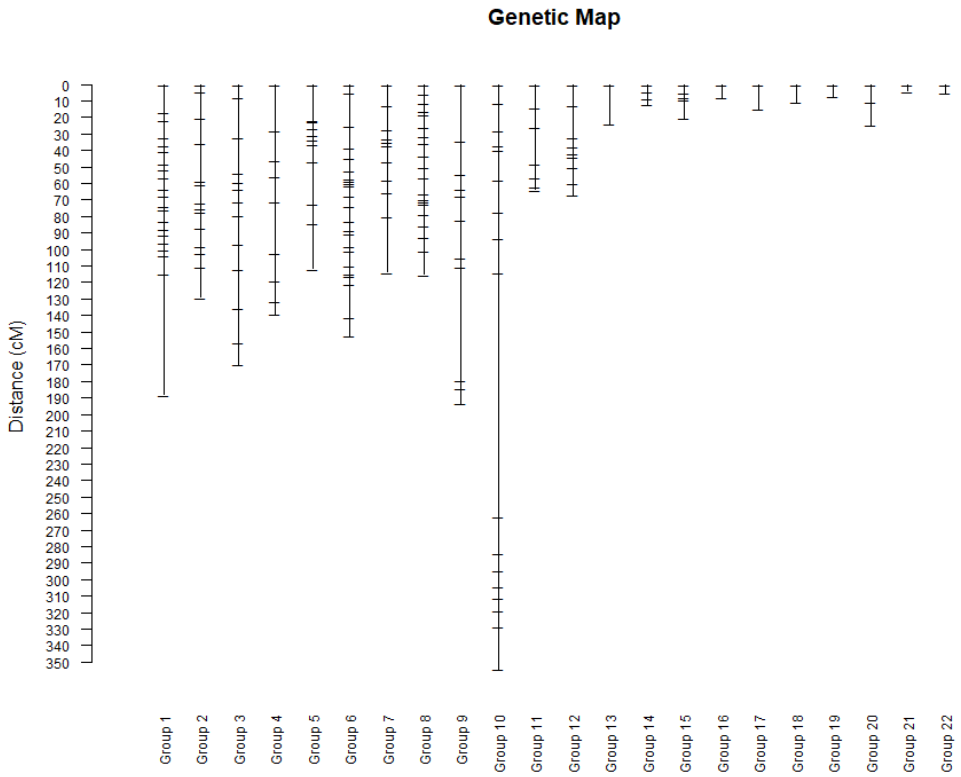

Figure 2- 13 Genetic linkage map of F2 mapping population 1063 with 273 individuals and 63 SNP markers (LOD = 5, recombination frequency = 0.4).

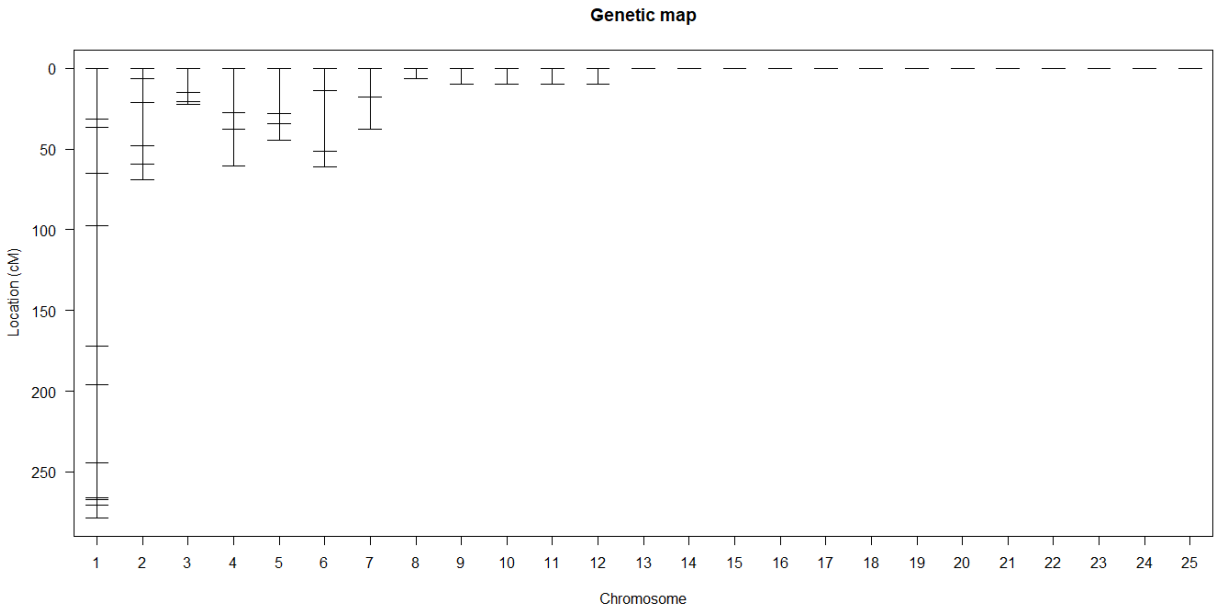

Figure 2- 14 Genetic linkage map of F2 mapping population 1060 with 84 individuals and 69 SNP markers (LOD = 12, recombination frequency = 0.3).

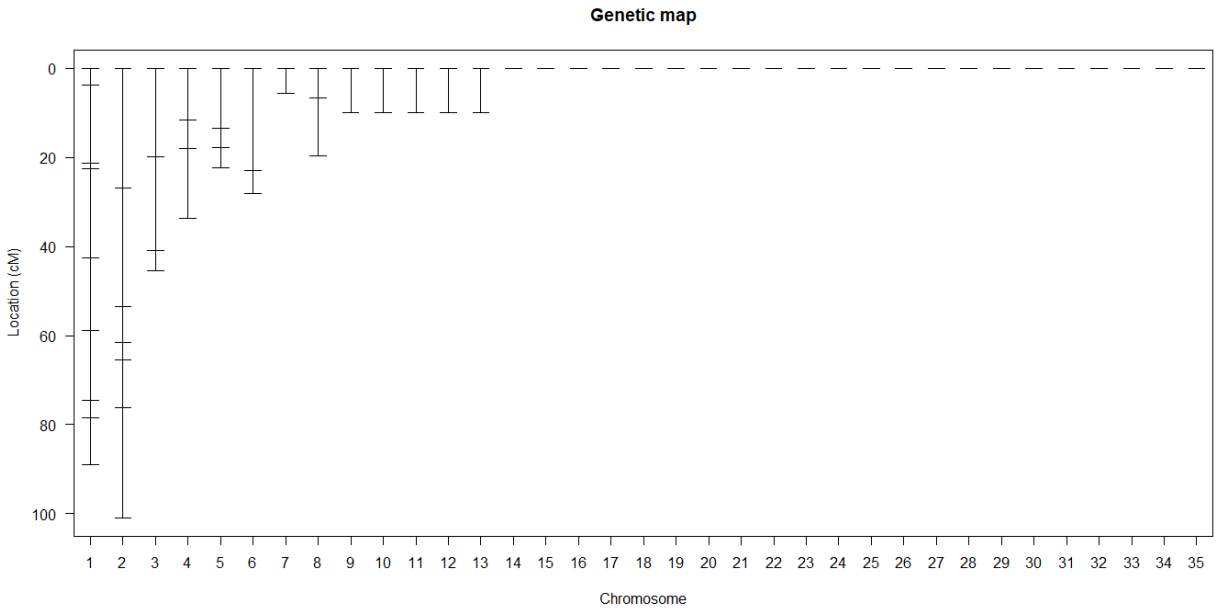

Table 2-1 Basic statistics of phenotypic data in the F1 mapping population 1025

Isolate	Sample Size	Mean	Variance	Std.Error	Skewness	Kurtosis	Minimum	Maximum
S1	186	0.6717	0.4728	0.05	1.1467	1.184	0	2.82
S3	91	0.4527	0.1977	0.04	1.037	0.1249	0	1.5319

Table 2-2 Putative QTL in the F1 mapping population 1025.

Isolate	Linkage group	Position (cM)	Left Marker	Right Marker	LOD	PVE (%)	LeftCI	RightCI
S1	1	197	SCAFFOLD_1252928_266	SCAFFOLD_51453_1155	7.8519	2.3928	191.5	200.5
S1	2	48	SCAFFOLD_24589_2109	SCAFFOLD_161603_938	12.0164	3.8803	45.5	48.5
S1	3	59	SCAFFOLD_363762_540	SCAFFOLD_481335_370	7.121	2.2985	55.5	61.5
S1	4	83	SCAFFOLD_379503_692	SCAFFOLD_68807_501	13.675	4.5124	82.5	86.5
S1	5	57	SCAFFOLD_36629_930	SCAFFOLD_183564_549	20.5745	8.8656	51.5	62.5
S1	5	72	SCAFFOLD_114063_1298	SCAFFOLD_280862_865	7.1494	3.2818	70.5	73.5
S1	7	36	SCAFFOLD_63297_1335	SCAFFOLD_755839_271	12.2484	4.314	32.5	38.5
S1	8	67	SCAFFOLD_2823_4049	SCAFFOLD_131199_1195	13.685	4.702	65.5	70.5
S1	8	141	SCAFFOLD_229673_556	SCAFFOLD_23348_66	7.9777	2.37	137.5	141
S1	9	164	SCAFFOLD_27772_1452	SCAFFOLD_91331_428	8.8239	2.743	159.5	165.5
S1	11	4	SCAFFOLD_91539_645	SCAFFOLD_2309241_103	8.5421	4.098	2.5	6.5
S1	16	0	SCAFFOLD_2361750_229	SCAFFOLD_16931_2930	12.0422	3.821	0	0.5
S3	1	283	SCAFFOLD_2727943_129	SCAFFOLD_289773_587	12.6691	19.161	279.5	289.5
S3	4	83	SCAFFOLD_379503_692	SCAFFOLD_68807_501	7.1916	7.775	81.5	85.5
S3	8	78	SCAFFOLD_69776_2168	SCAFFOLD_1336152_191	12.2527	14.870	75.5	79.5
S3	13	8	SCAFFOLD_3297248_113	SCAFFOLD_47202_1495	6.8375	10.209	2.5	13.5

Table 2-3 Basic statistics of phenotypic data in the F2 mapping population 1063.

Isolate	Sample Size	Mean	Variance	Std.Error	Skewness	Kurtosis	Minimum	Maximum
PP8	242	0.6746	0.553	0.047	2.65	9.32	0	4.73
UH2	190	0.7227	0.519	0.052	1.97	3.66	0	3.65

Table 2-4 Basic statistics of phenotypic data in the F2 mapping population 1060.

Isolate	Sample Size	Mean	Variance	Std.Error	Skewness	Kurtosis	Minimum	Maximum
RP8	77	0.9177	0.023	0.0175	-0.951	2.51	0.28	1.25
UH2	65	0.8551	0.016	0.0160	-1.034	2.54	0	1.13

CHAPTER 3: EVALUATION OF SEGREGATING TARO PROGENIES FOR YIELD

Abstract

Yield is one of the important criteria used for selection in a taro breeding program. Each cross-pollination of the flowering structure could produce thousands of taro seedlings and initial evaluation of the offspring is done in un-replicated trials where only a few check cultivars are replicated. This leads to a poor estimation of the residual variances. The hierarchical Bayesian G ×E model is designed to deal with the unbalanced data from a large number of trials. In this study, a particular genotype 1025-181 was found promising for high yield and stable performance. Genotypes 1016-13, 1024-215 and 1025-281 were also found to be good yielding and highly resistant to the Taro Leaf Blight (TLB) disease. These genotypes should be incorporated in the breeding program and further selection should be made. This method can be used to evaluate the genotypes in future trials.

Introduction

Taro is grown mostly for corm production, which is a primary source of calories for people in the tropics. Corms are an excellent source of starch and can be used as a source of carbohydrates in many countries (Simsek, 2012). Boiled taro corms have the same energy value as rice and exceeds the energy value of beans (Guchhait et al., 2008). Taro can be grown in both lowland (i.e., wetland) and upland (i.e., dryland) conditions. However, it requires irrigation or reliable rainfall to grow in the non-flooded conditions. In Hawaii, historically most of the taro was grown in a submerged land with heavy clay loam or sandy loam soil (Parris, 1941). Upland taro production has great potential in Hawaii, because of the increased availability of land due to the cessation of sugarcane production as well as not requiring relatively scarce wetland areas with reliable water flow (Miyasaka et al., 2003; Teves, 2015).

Taro thrives in a warm, moist environment with well-distributed rainfall. The best soil for upland taro is well-drained, friable loamy soil with pH 5.5 to 6.5 (Kay, 1987). For submerged cultivation, heavy soil like clay is preferable as clay helps to hold the water near the soil surface (Teves, 2015). Water availability is more important than soil type for taro grown in upland soil and it can drastically affect the yield (Parris 1941). Taro can be planted in furrows or on ridges between furrows and in a flat land system (Teves, 2015).

Weed control is crucial for taro growth and development as it cannot compete with the weeds during the initial months of growth in the field until the canopy closes. Dense plantings of taro could improve weed control, but this causes poor air circulation and increased chances for proliferation of disease and pests. High-density plantings could also lead to the rapid spread of Taro Leaf Blight (TLB) disease, the most destructive disease caused by *Phytophthora colocasiae*. In a hectare of land, the ideal plant density is 17290 plants. The spacing generally ranges from 40-

60 cm (within a row) and 90-180 cm (between row) (Teves, 2015). Fertilizer application is done prior to planting and over the cropping season.

Organic and inorganic fertilizers are incorporated in the soil during land preparation. Taro can be ready to harvest between 6 to 18 months but the indicators used for crop maturity could be different because of the indeterminant growth of taro (Singh et al.,1998). Crop maturity of taro could be defined based on average or maximum yield, acceptable quality, dry weight of corm and cormels, and development stage of the leaf (Miyasaka et al., 2003).

Taro is highly heterozygous and selections could be made directly in the F_1 generation. The most common breeding method in taro is cross pollination of parental genotypes to develop progenies that segregate for different traits. Selected individuals from segregating progenies are subjected to field trials where they are compared against the standard cultivar (high yielding, commercial type) (Lebot et al., 2000). Once a desirable individual is found, then clonal propagation is conducted to stabilize the trait.

The other common breeding method in taro is backcross breeding, where the objective is to incorporate a single trait into a good cultivar. A modified backcross breeding strategy is often utilized by breeders to include positive alleles from several cultivars with desirable traits into a single cultivar. In modified backcross breeding, more than one recurrent parent is used to avoid inbreeding depression.

Selection for quantitative traits like yield is done by growing high yielding clones in replicated trials in many locations. The chances of finding a high yielding clone, with disease resistance and good taste quality in a conventional breeding program are very low (Mackay, 2003).

A breeder needs to evaluate many genotypes to identify good yield and good taste in the same clone (Lebot et al., 2000).

A major goal for breeders is to develop cultivars suitable to local agroecosystems (Rivière et al., 2015). One way to do this is to include farmers in the breeding programs, so they could contribute to the development of breeding objectives and statement of needs. In this method, the selection is done in target environments or on-farm. In such trials, many genotypes are included with very few replications. The amount of plant materials for multi-location trials is hard to achieve because the multiplication rate of taro is very low (Lebot et al., 2000). Only standard cultivars are replicated and comparison of breeding cultivars is made with them. The objective of this study was to evaluate genotypes that are developed from the ongoing breeding program at the University of Hawai‘i at Mānoa for yield to identify best performing individuals and advance them for future breeding.

Materials and Methods

Progenies of different crosses that are segregating for TLB resistance were planted in field trials at (19.834964°N, 155.100767°W) and (19.835211°N, 155.115287°W) in Pepeekeo, Hilo. In addition to TLB resistance, these cultivars were evaluated for yield and quality attributes. Data on yield, corm rot and dry matter of corms were available for five years (2013-2018). Every year, new genotypes were added from the breeding program. Two-hundred and ninety-five genotypes were included in the yield data over this time frame. Only check cultivars Maui Lehua and Bun Long were replicated each year. Most of the genotypes were replicated few times or not at all (Figure 3-1). This lead to the poor estimation of residual differences and statistical differences of mean. In addition, the yield data were analyzed by Multiple Comparison with the Best (MCB) in SAS and PPBstats in R.

Multiple Comparison with the Best (MCB)

Data from genotypes that were present at least twice out of the six years were subjected to the Multiple Comparison with the Best procedure for mean separation in SAS (version 9.4; SAS Institute, Cary, NC). MCB is similar to Dunnett's method except that it suggests a control "best" itself. The best is unknown but conceptualized as the sample with highest μ. This method develops a joint confidence interval for the vector of differences from the unknown best samples parameter. The idea is that multiple best can exist at a specified level of confidence. It calculates the upper and lower confidence value on the deviation of all the sample values from the best value (Horrace et. al., 2000). MCB categorizes the samples into two groups; a) group that has samples that are not significantly different than the best but are significantly better than in other group and b) group that contains samples that are significantly different than the best. This method reduces the number of comparison to be done and it increases the power of the analysis by controlling the experiment-wise error and by preventing the reduction in individual significance levels (Miyasaka, 2012; Taylor, n.d.).

Prior to that, an ANOVA analysis was performed to see whether genotypes are significant for the variables. Since, genotypes were temporally replicated, year was used as a blocking factor in the model. The model used is

$$Y_{ij} \sim \text{Genotype}_{ij} + \text{Year}_{j} + \text{Residual}\ [\varepsilon_{ij} \sim N(0, \sigma^2)]$$

Where, Y_{ij} is the independent variable yield, rot or dry matter for i^{th} genotype in j^{th} year.
Genotype_{ij} is the i^{th} genotype in j^{th} year.
Year_{j} is the j^{th} year.
$\varepsilon_{ij} \sim N(0, \sigma^2)$ indicates that the residuals are normally distributed.

Exploring the data in Bayesian Context

The R package (PPBstats v0.23) was developed by Rivière et al. (2015, 2018), that compares genotypes from on-farm trials which are highly unbalanced). Data were formatted as Incomplete Block design (IBD) in which entries are not replicated, but some entries are shared by some locations.

This method in PPB stats (Riviere et al., 2015) is based on a Bayesian model:

$Pr(\theta|y) \propto Pr(\theta)Pr(y|\theta)$ with $Pr(\theta|y)$ the posterior, $Pr(y|\theta)$ the likelihood and $Pr(\theta)$ the prior.

The distribution of the parameter of interest is proportional to the distribution of the prior × the information brought by the data. For mean comparison, the residual variance of a trial is estimated using all information available from total data (including all trials) rather than using data from that trial only. Therefore, the mean of each entry is compared to the mean of each other entry.

$$H_0: \mu ij \geq \mu i'j$$

$$H_1: \mu ij < \mu i'j$$

if either H_0 or H_1 had a high posterior probability. If $Pr\{H0|y\} > 1 - \alpha$ or $Pr\{H1|y\} > 1 - \alpha$, where α was some specified threshold, the difference ($\mu ij - \mu i'j$) between the means of germplasm i and population i' in environment j was considered as significant. The difference was considered as not significant otherwise. Groups are made based on the posterior probabilities. This threshold is set by default to $\alpha = 0.1/I$ (with I the number of entries in each environment). Further, PPBstats (Riviere et al., 2015) also allows detection of the environment where certain genotypes performed the best. This analysis compares the importance of germplasm effect *vs* (germplasm*environment) interaction effect. Higher interaction effect means the trait is highly affected by the growing environment. If the germplasm effect is greater than the interaction effect, then selection of the

germplasm based on mean performance is meaningful for that environment. For mean comparison, the mean of each entry is compared to the mean of every other entry.

Model 1 was used to compare the mean of genotypes within a single year. It assumes that the trial residual variance comes from a common distribution because of the similar structure of the trials. The model can be described as

$$Y_{ijk} = \mu_{ij} + \beta_{jk} + \varepsilon_{ijk};\ \varepsilon_{ijk} \sim N(0, \sigma j^2),$$

Where,

Y_{ijk} is the phenotypic value of a genotype i in block k and environment j for a variable Y.
μ_{ij} is the mean of germplasm i in environment j.
β_{jk} is the effect of block k in environment j.
ε_{ijk} is the residual error.
σj^2 is the variance specific to environment j and is calculated using the information from all trials.

Model 2 within PPB stats (Bayesian hierarchical model G×E) was used to study the performance of the genotypes over different years. It estimates the germplasm and location and interaction effect. The model can be described as

$$Yij = \alpha i + \theta j + \eta i\theta j + \varepsilon ij;\ \varepsilon ij \sim N(0, \sigma^2_e)$$

Where,

Yij is the phenotypic value of a genotype i in environment j for a variable Y.
αi is the main effect of germplasm i.
θj is the main effect of environment j.
$\eta i\theta j$ is the interaction effect between genotype i and environment j.
εij is the residual error.

Results

MCB

One-way ANOVA analysis identified significant difference between genotypes for yield and dry matter content. Average rot was not significant (Table 3-1). The model showed a better fit with dry matter data compared to the yield data (Figure 3-2). However, the data was not normally distributed for both variables. Since, MCB is based on the fixed-effect, the distribution assumption of mean is not terribly important (Fraser et al., 2003). The data on average yield and dry matter content were successfully analyzed and two groups were identified in both cases. The results for average yield suggested that genotype 1025-181 has the highest mean for average yield and genotype 1014-13 has the highest percent dry matter. Seven other genotypes were not different than 1025-181 in terms of yield; these were 1016-3, 1016-19, 1024-209, 1024-215, 1025-181, 1025-269, 1025-133 and 1025-65. However, the group was relatively large for dry matter content; 113 genotypes were similar to the best genotype 1014-13; the eight genotypes identified as highest yielding also did not differ from the best in percent dry matter. Out of these eight genotypes that were significantly higher in yield and percent dry matter, four were from the 1025 population and two each from the 1016 and 1024 population (Table 3-2).

Bayesian Modeling of Yield components

In this study, six years (2013 to 2018) one location (Hilo, Hawaii) were used in the analysis. Genotypes that were not present in at least two years were dropped from the model, because they were either highly susceptible to diseases (TLB and/or corm rot) or produced undesirable rhizomes (i.e., runners) or excess cormels. A few potential superior genotypes for breeding were identified in this study.

Within year variation in genotypes for yield

The residuals were normally distributed for model 1 (Figure 3-3). Mean comparison was done at alpha = 0.05 and soft Bonferroni correction was conducted. The $\alpha = 0.1/I$ (with I the number of entries in each environment) was used as soft Bonferroni correction instead of the Bonferroni Correction. mean comparison of genotypes for each year identified different groups. In year 2013, 145 genotypes were included in the analysis. No significant differences were observed between genotypes (Appendix 2A). In the year 2014, twenty-one genotypes were present. There were no significant differences between genotypes (Appendix 2B). In the year 2015, there were 149 genotypes and significant differences were found between genotypes. Genotype 1025 – 181 had a significantly higher yield than others (Figure 3-4). No groups were formed for the year 2016 because the model didn't identify the control. The control cultivar 'Maui Lehua' was present only once in the year 2016. Year 2017 had 59 genotypes and no significant differences were found between genotypes (Appendix 2C). In year 2018, there were 88 genotypes in year 2018, and no significant difference between genotypes were found (Appendix 2D).

Genotypic effect

Mean comparison of genotypes for six years of trials

Within the model 2 (PPB stats), the standardized residuals follow a normal distribution, indicating a good fit of the model (Figure 3-5). The Bayesian G×E model categorized the genotypes into different groups. This categorization was based on the genotypic effect on average yield. The parameter used here is α_i, and it is not simply the arithmetic mean of the data. This parameter is calculated using the main effect of the genotype considering the sensitiveness of the genotype in the environment. There was no significant difference between genotypes at Type I error = 0.5 (Appendix 3).

Genotype × Environment interaction

The alpha-beta biplot shows the G×E interaction for each genotype (Figure 3-6). Alpha is the genotypic effect on the yield. Beta is the sensitivity of a genotype to environments. Higher values of beta_i indicate high environmental effect on the phenotype. A higher environmental effect is not good for selection because a genotype performing good in one location/year could be a poor performer in other environments. The results from the biplot shows that 1025-181 (germ:106) has a high genotypic effect and low environmental effect. Genotype 1025-124 and genotype 1025-281 have a low environmental effect on the yield (i.e. yield is stable over years). These two genotypes also have a better genotypic value compared to the many other genotypes.

Discussion

Participatory plant breeding (PPB) approach is different than conventional breeding in that farmers are included in the breeding process and collaboration exists between the researcher and a farmer. The cultivars selected from a breeding program should meet the need of the farmer and thus requires involving farmers in the decision-making process such as defining the objectives, in on-farm evaluation and selection process (Dawson et al., 2012). A participatory approach is more popular in developing countries where farmers cannot modify the growing environment but can use varieties that are more suitable to their local environments, benefiting from the genotype × environment interaction (Ceccarelli et al., 2009). The adaptation of cultivars from a breeding program is higher in PPB because a local landrace or locally adapted variety is used in the breeding program. Ceccarelli et al. (2000) found that the selection criteria used by farmers were congruent with that of the breeders, and that farmers were more effective than breeders in identifying high yielding entries in their own field. Decentralized breeding is very

useful to minimize the need for multi-location and highly replicated trials and the importance is more pronounced in case of marginalized and neglected crops.

The best results from analyzing the unbalanced data using Bayesian hierarchical model comes from many environments (location × year) (Rivière et al., 2015). Genotypes that are consistent over multiple environments are desirable in breeding programs and are good breeding stock for the future. From the PPBstat results, genotype 1025-181 had higher yield in year 2015 and highly consistent over four years. However, selection in taro is not only based on the yield. There are other desirable factors include disease resistance, optimum taste qualities, the absence of rhizomes, optimum number of cormels, and most importantly in Hawaii, the suitability for making poi. For poi, this corm characteristic includes high stickiness (i.e, gumminess) and desirable taro flavor. Considering these factors, several other genotypes could be used also for further study, including genotype 1016-3, genotype 1024-215and genotype 1025-281 that are all highly resistant to TLB disease and have good yield compared to the commercial cultivar genotype 1 (Maui Lehua). The results from MCB also supports these genotypes for good yield and high dry matter content. Seven genotypes were identified from the MCB analysis to have high yield and better dry matter.

Conclusion

Highly unbalanced data from taro trials were evaluated using the MCB method and Bayesian model. The results from the two different methods was found to be similar. The MCB method was more powerful in identifying significance, however, the ability to study the effect of environment and interaction was compromised. Although multiple best genotypes were identified using MCB, they could not be further differentiated and the ranking of the genotypes could not be done. The other limitation of MCB is that it is unclear what is causing the

differences between the groups. This method is useful in order to identify breeding lines that could be used for further improvement, however we cannot recommend a genotype to a farmer based on these results. On the other hand, the Bayesian approach was limited in telling the significant differences in means based on the genotypic effect but at the same time allowing us to make selection decisions with information on environmental effect. Adding more data from future trials would improve the model efficiency. The Bayesian approach results shows that genotype 1025-181 was found to have a high yield and performed well in multiple years. The potential superior genotypes identified from this study could be further utilized in the taro breeding program. Important information on Genotype × Environment interaction from this evaluation could lead to better selection. Final selections should be made based on yield attributes as well as other important criteria. An ideal cultivar would have high yield, desirable taste, improved resistance to disease, and good plant architecture. Oftentimes it is very challenging to have all the desirable characters in one cultivar because of a trade-off between them. It might require longer cycles of breeding to get rid of the undesirable characteristics. Thus, breeding is a continuous process of cultivar improvement in taro.

Simple changes in the trial design in future could led to the efficient analysis of breeding populations. Including the standard cultivar Maui Lehua in each row in the field trial will allow us to get more accurate estimation of the trial variances. This will also give us more flexibility in analyzing the data using different models.

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Tables and figures

Figure 3- 1 Presence-absence plot for average yield variable in six years. The nb_measures indicate the number of time a germplasm is replicated over different years.

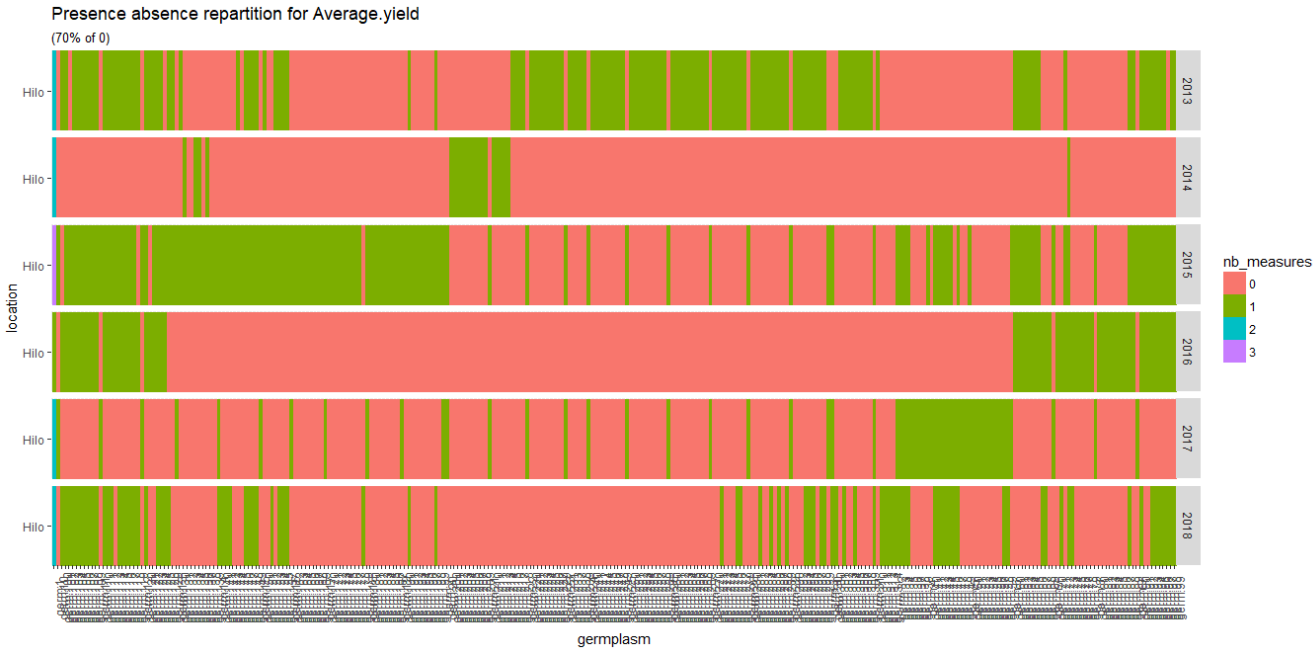
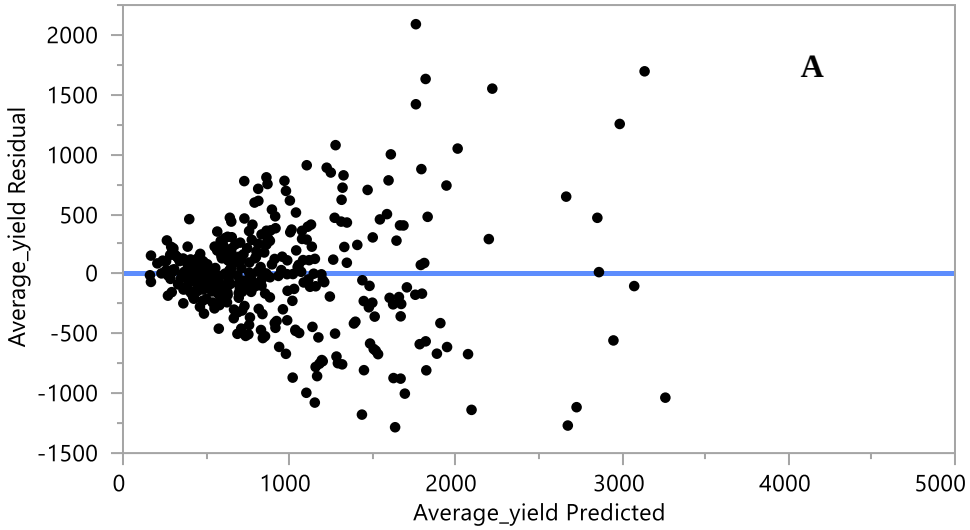


Figure 3- 2 Residual by Predicted Plot for A) Average yield and B) Percent Dry matter using the linear model = y ~ Genotype + Year, where y is the outcome variable. The genotypes are replicated in time and year is used as a blocking factor in the model.



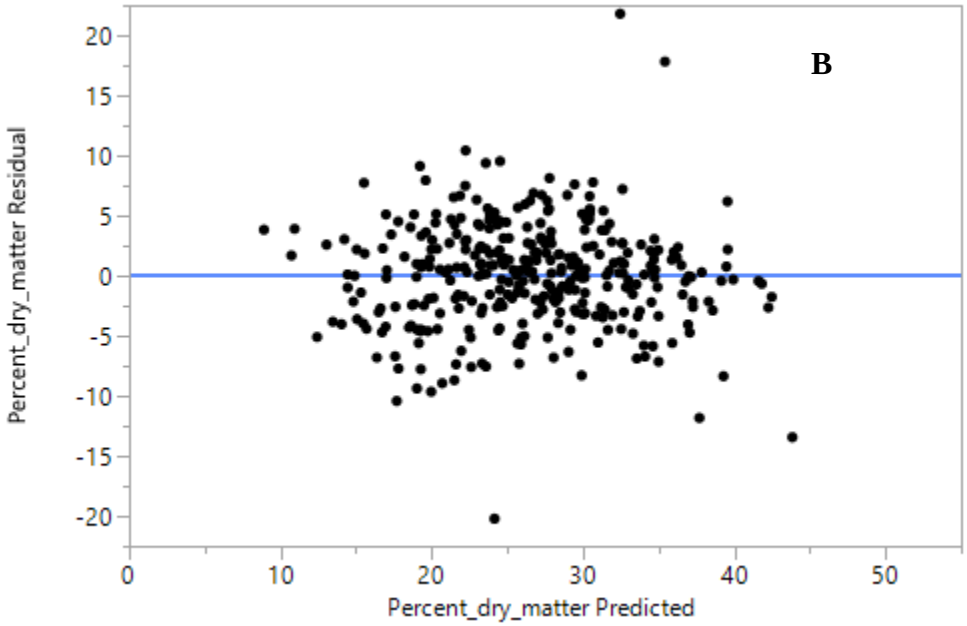


Table 3-1 Type of test 3 fixed effect results for average yield, average rot and dry matter. It is a hypothesis test for each fixed effect specified in the model, in this case we are interested in the fixed effect genotype.

Average yield

Effect	Degree of Freedom	DFFValue	Pr > F
Genotype	131	2322.56	< ·0001

Average rot

Effect	Degree of Freedom	DFFValue	Pr > F
Genotype	131	2281.14	0.1905

Dry matter

Effect	Degree of Freedom	DFFValue	Pr > F
Genotype	131	2221.58	0.0016

Table 3-2 Pedigree information, Fresh weight and Dry matter content of the corms of 132 taro genotypes that were planted in six years. "Multiple Comparison with the Best" identified two categories, a) those not different from the best or maximum value and b) those that are different from the best. The Fresh weight and Dry matter values are highlighted when they are not significantly different from the best. Genotypes that have high fresh weight and high dry matter are highlighted as well.

Name of the genotype	Pedigree/Cultivar Name	Fresh weight (grams) Mean±SE	Dry matter Mean±SE (%)
176	Maui Lehua	739.34±231.77 b	19.5987±3.1836 b
74	Kalalau	452.2±401.43 b	16.723±5.0337 b
75	Hapuu	321.43±401.43 b	11.1875±5.0337 b
79	Mana Eleele	550.13±401.43 b	**20.78±5.0337 a**
84	Pololu	653.79±401.43 b	**36.865±5.0337 a**
5	Bun Long	584.49±327.77 b	**25.2413±4.11 a**
230	Moi X P20-7	735.78±401.43 b	**26.5606±5.0337 a**
254	Eleele NaioeaXWhite Bun Long	512.14±401.43 b	**28.1987±5.0337 a**
255	[Red Moi X PH15]XSawahn Kurasae	499.97±401.43 b	**30.1428±5.0337 a**
37	Lauloa Eleele-ula	525.51±401.43 b	17.973±5.0337 b
38	Lauloa Palakea-eleele	700.46±401.43 b	16.318±5.0337 b
39	Lauloa Palakea-ula	545.43±401.43 b	**20.9105±5.0337 a**
47	Nawao	345.92±401.43 b	15.3245±5.0337 b
55	Manini Toretore	358.96±401.43 b	**22.064±5.0337 a**
24	Uahiapele	332.74±401.43 b	14.6995±5.0337 b
58	Nihopuu	737.63±401.43 b	**19.2385±5.0337 a**
59	Manini-opelu	520.24±401.43 b	**18.9955±5.0337 a**
62	Ohe	230.74±401.43 b	**24.2295±5.0337 a**
68	Wehiwa	293.45±401.43 b	17.599±5.0337 b
80	Mana Okoa	450.72±401.43 b	**19.9665±5.0337 a**
88	Makalau (Molokai)	321.38±401.43 b	16.72±5.0337 b
4	Iliaua	524.79±401.43 b	**22.2595±5.0337 a**
86	Red Moi	587.58±401.43 b	**20.9755±5.0337 a**
87	Kai KBS	224.54±401.43 b	**22.8245±5.0337 a**
89	Keone (melim)	483.1±401.43 b	**26.0915±5.0337 a**
46	Kumu-eleele	384.92±401.43 b	**21.2985±5.0337 a**
142	Ka'ano'i	490.71±327.77 b	14.902±7.1188 b
159	Purple Manalud	617.19±327.77 b	NA
188	Niumalu Luau	1679.13±327.77 b	**28.7303±4.11 a**
199	Niumalu X2	1564.84±327.77 b	19.8027±4.11 b
200	Fifty Baby	548.09±327.77 b	**25.2556±4.11 a**
224	Okinawa	649.52±327.77 b	**30.31±4.11 a**
48	Ulaula Kumu	370.73±401.43 b	13.213±5.0337 b
228	Moi X P20-2	882.85±327.77 b	**32.794±4.11 a**
229	Moi X P20-6	655.73±327.77 b	**22.3488±4.11 a**

245	Unknown	750.41±327.77 b	**21.8661±4.11 a**
246	False wehiwa	708.28±327.77 b	**30.8965±4.11 a**
247	Lehua Maoli X PRG 263	368.93±327.77 b	**26.6633±4.11 a**
248	Niumalu X P10	684.98±401.43 b	**21.4358±5.0337 a**
257	Maui Lehua X P5	353.4±327.77 b	NA
50	Ulaula Moano	402.96±401.43 b	17.346±5.0337 b
70	Kuoho	453.93±401.43 b	15.3634±5.0337 b
110	A'alii	465.02±401.43 b	16.6618±5.0337 b
51	Niue-ulaula	359.49±401.43 b	**21.4215±5.0337 a**
54	Manini Kea	362.52±401.43 b	**21.5265±5.0337 a**
56	Papakolea-koae	268.89±401.43 b	**18.8935±5.0337 a**
73	Haokea	498.65±401.43 b	17.841±5.0337 b
1003-3	[Moi x P20-1] X '02-57	923.62±401.43 b	**22.0167±5.0337 a**
1005-18	230 X 255	1257.07±401.43 b	28.1696±5.0337 a
1005-35	230 X 255	1090.84±401.43 b	22.8252±5.0337 a
1007-110	2000-8 X Lauloa Palakea-eleele	1083.76±401.43 b	**25.1405±5.0337 a**
1010-16	Maui Lehua X 255	716.27±327.77 b	**29.3716±4.11 a**
1011-5	255 X [Maui LehuaXPwetepwet(Guam)]	1614.56±327.77 b	**32.5356±4.11 a**
1014-39	Pa'lehua(192) X Maui Lehua	1673.09±327.77 b	**38.5296±4.11 a**
1016-3	[(Red MoiXPH15)Xvanuatu 4] X kai UliUli	**2839.58±327.77 a**	**23.0808±4.11 a**
1016-19	[(Red MoiXPH15)Xvanuatu 4] X kai UliUli	**2073.79±327.77 a**	**24.2749±4.11 a**
1024-209	255 X 230	**1730.81±401.43 a**	**29.6212±5.0337 a**
1024-215	255 X 230	**1839.89±327.77 a**	**30.723±4.11 a**
1025-120	230 X 255	1057.75±327.77 b	**30.0028±4.11 a**
1025-124	230 X 255	1469.7±283.85 b	**27.3554±3.5594 a**
1025-129	230 X 255	1018.72±327.77 b	**25.5414±4.11 a**
1025-174	230 X 255	929.93±283.85 b	**36.1309±3.5594 a**
1025-175	230 X 255	1314.08±283.85 b	**30.5087±3.5594 a**
1025-180	230 X 255	1161.25±283.85 b	**28.9247±3.5594 a**
1025-181	230 X 255	**3103.43±283.85 a**	**24.989±3.5594 a**
1025-186	230 X 255	1135.46±283.85 b	**28.7742±3.5594 a**
1025-239	230 X 255	1107.35±283.85 b	**32.8865±3.5594 a**
1025-242	230 X 255	1356.55±283.85 b	**30.3089±3.5594 a**
1025-250	230 X 255	884.91±283.85 b	**27.1415±3.5594 a**
1025-255	230 X 255	620.13±283.85 b	**23.4797±3.5594 a**
1025-269	230 X 255	**2042.43±283.85 a**	20.5118±3.5594 b
1025-274	230 X 255	450.69±327.77 b	**23.7306±4.11 a**
1025-281	230 X 255	1280.01±283.85 b	**30.2083±3.5594 a**
1025-283	230 X 255	394.8±283.85 b	**27.3071±3.5594 a**
1025-288	230 X 255	671.05±283.85 b	**29.0237±3.5594 a**
1025-297	230 X 255	1640.88±283.85 b	**21.1694±3.5594 a**
1025-299	230 X 255	1674.8±283.85 b	**22.0661±3.5594 a**
1025-312	230 X 255	836.53±327.77 b	**25.1626±4.11 a**

1025-317	230 X 255	800.87±283.85 b	**25.5032±3.5594 a**
1025-332	230 X 255	1016.78±401.43 b	**26.0274±5.0337 a**
1025-350	230 X 255	915.75±327.77 b	**28.8009±4.11 a**
1025-377	230 X 255	902.75±283.85 b	**27.9329±3.5594 a**
1025-399	230 X 255	904.4±327.77 b	**27.7714±4.11 a**
1025-509	230 X 255	754.87±327.77 b	**29.5753±4.11 a**
1025-511	230 X 255	968.23±327.77 b	**31.8418±4.11 a**
1025-379	230 X 255	642.12±401.43 b	**24.0675±5.0337 a**
1025-406	230 X 255	1215.08±401.43 b	**25.6425±5.0337 a**
1025-96	230 X 255	1063.94±401.43 b	**23.7714±5.0337 a**
1025-125	230 X 255	876.1±401.43 b	**26.3573±5.0337 a**
1025-168	230 X 255	644.81±327.77 b	**30.5548±4.11 a**
1025-187	230 X 255	466.02±327.77 b	**27.3012±4.11 a**
1025-291	230 X 255	709.32±327.77 b	**29.664±4.11 a**
1025-224	230 X 255	666.89±327.77 b	**23.1076±4.11 a**
1025-122	230 X 255	926.25±401.43 b	**27.2673±5.0337 a**
1025-278	230 X 255	638.69±401.43 b	**23.2784±5.0337 a**
1025-188	230 X 255	1430.4±401.43 b	**31.2937±5.0337 a**
1025-130	230 X 255	1174.1±401.43 b	**27.8511±5.0337 a**
1025-51	230 X 255	518.65±401.43 b	NA
1025-100	230 X 255	693.44±401.43 b	16.4191±5.0337 b
1025-482	230 X 255	458.85±401.43 b	**36.7841±5.0337 a**
1025-35	230 X 255	1024.47±401.43 b	**34.238±5.0337 a**
1025-302	230 X 255	445.68±401.43 b	**24.9693±5.0337 a**
1025-9	230 X 255	1290.85±401.43 b	**31.0736±5.0337 a**
1025-72	230 X 255	471.45±401.43 b	**24.5437±5.0337 a**
1025-225	230 X 255	554.4±401.43 b	**33.8772±5.0337 a**
1025-87	230 X 255	555.3±401.43 b	**29.0393±5.0337 a**
1025-113	230 X 255	324.03±401.43 b	**27.614±5.0337 a**
1025-133	230 X 255	**1843.57±327.77 a**	**35.6889±4.11 a**
1025-141	230 X 255	1292.73±327.77 b	**27.2017±4.11 a**
1025-146	230 X 255	592.41±327.77 b	**35.7565±4.11 a**
1025-161	230 X 255	1139.23±327.77 b	**22.524±4.11 a**
1025-164	230 X 255	444.88±327.77 b	**30.6768±4.11 a**
1025-210	230 X 255	1096.45±327.77 b	**28.6736±4.11 a**
1025-382	230 X 255	999.97±327.77 b	**22.2556±4.11 a**
1025-13	230 X 255	1018.35±283.85 b	**28.3475±3.5594 a**
1025-19	230 X 255	1428.9±327.77 b	**33.1501±4.11 a**
1025-56	230 X 255	621.39±283.85 b	**25.6578±3.5594 a**
1025-65	230 X 255	**2746.67±327.77 a**	**27.7641±4.11 a**
1025-74	230 X 255	778.74±327.77 b	**32.4928±4.11 a**
1025-79	230 X 255	419.8±283.85 b	**22.6545±5.0337 a**
1025-81	230 X 255	880.11±283.85 b	**36.5602±3.5594 a**

1025-82	230 X 255	1654.48±283.85 b	**32.618±3.5594 a**
1025-91	230 X 255	726.81±283.85 b	**31.5613±3.5594 a**
1025-99	230 X 255	757.21±327.77 b	**27.0819±4.11 a**
1025-111	230 X 255	597.66±283.85 b	**35.9124±3.5594 a**
1025-118	230 X 255	1633.6±283.85 b	**30.8098±3.5594 a**
1027-2	[MoixP20-1] X Eleele Naioea	417.58±401.43 b	**28.4125±5.0337 a**
1027-337	[MoixP20-1] X Eleele Naioea	839.68±401.43 b	**26.4485±5.0337 a**
1027-341	[MoixP20-1] X Eleele Naioea	570.26±401.43 b	**28.806±5.0337 a**
1027-133	[MoixP20-1] X Eleele Naioea	647.17±283.85 b	**24.4175±3.5594 a**
1028-137	[MoixP20-1] X Piko Eleele	609.48±401.43 b	**25.459±5.0337 a**
1032-1	1016 X {[Maui LehuaXPwetepwet(Guam)] X Lauloa Keokeo	825.41±401.43 b	**25.5471±5.0337 a**

Figure 3- 3 Distribution of the standardized residuals for model which explore the within year variation between genotypes. The predicted average yield values are given in the x-axis and the residuals are plotted in the y-axis. The model went well as all the residuals lie between -2 and 2.

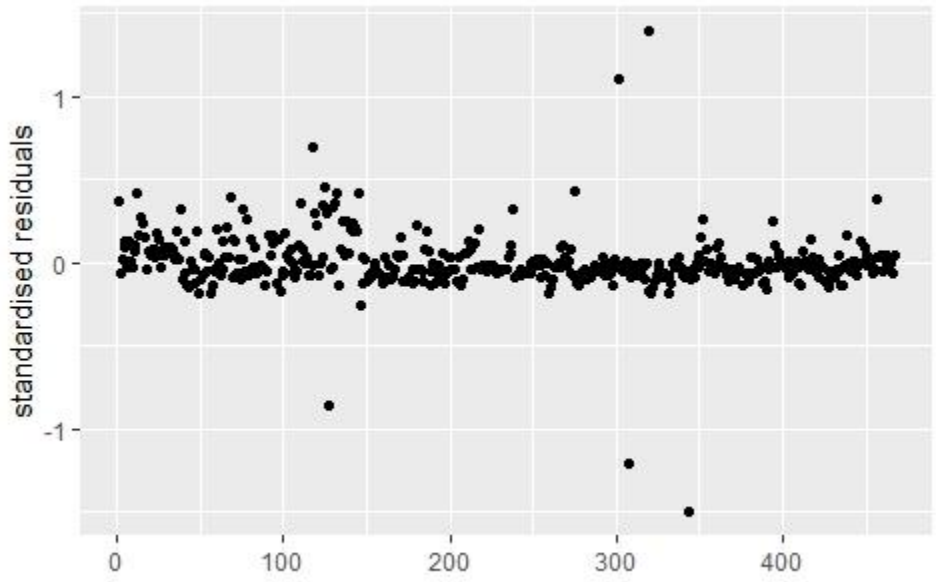


Figure 3- 4 Mean comparison of the genotypes using model 1 for the year 2015. Model 1 is used to explore the variation between genotypes within a year. Bonferroni correction at alpha = 0.5 was used. Genotypes with different letters are significantly different. Genotype 1025-181 has significantly higher yield than others in the year 2015. Only 12 genotypes are shown.

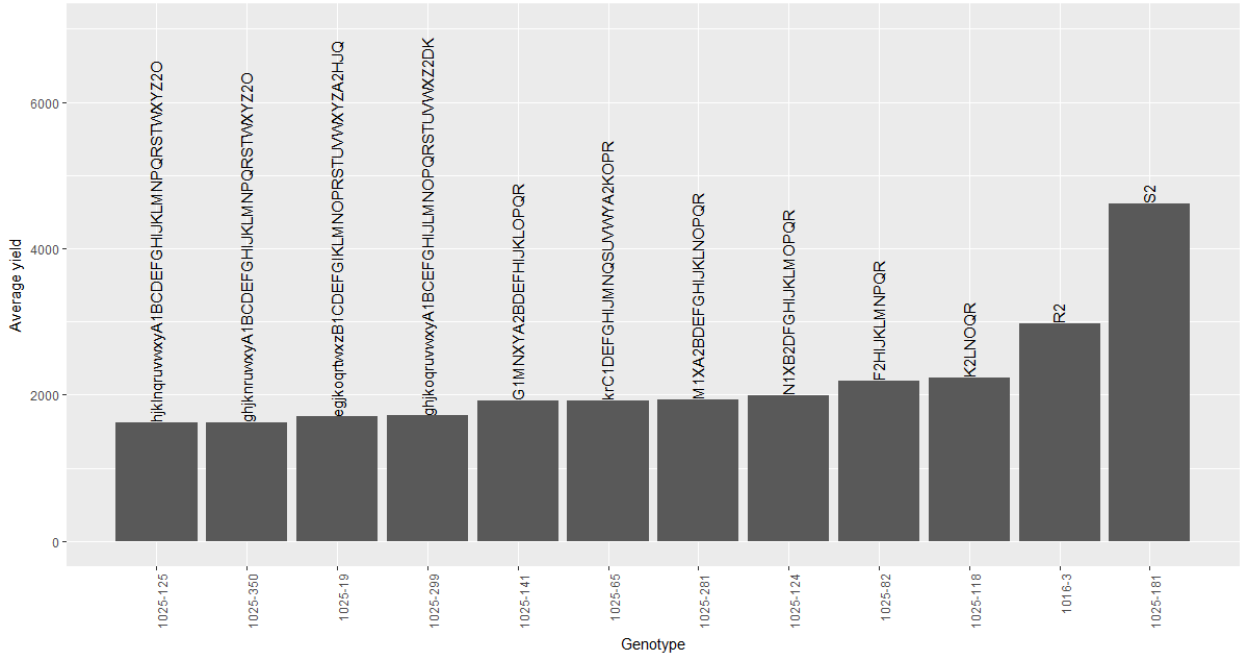


Figure 3- 5 Distribution of the standardized residuals for Bayesian G×E model. Bayesian G×E model uses the information from all years to estimate the overall variation between the genotypes. The model fit well as all residuals lie between -2 and 2 with exception of few outliers.

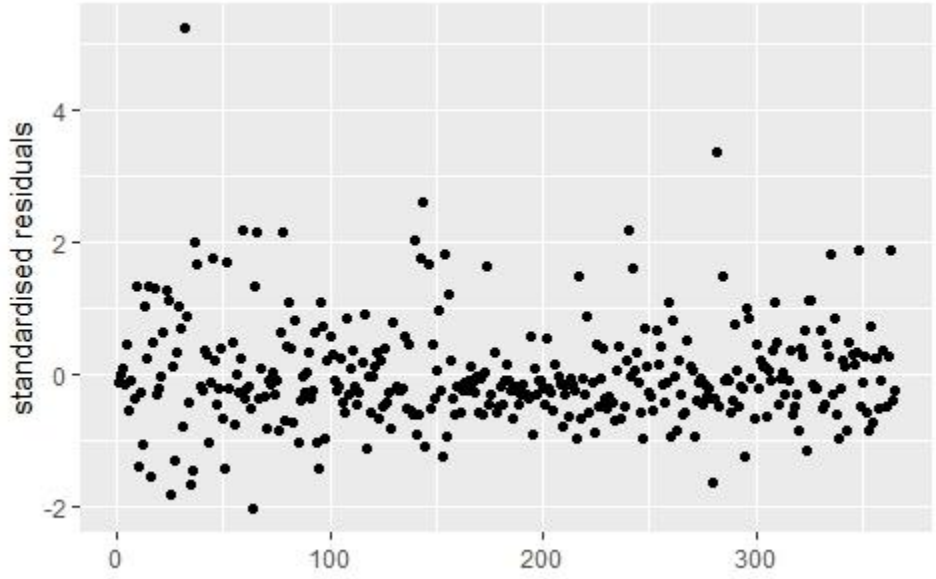
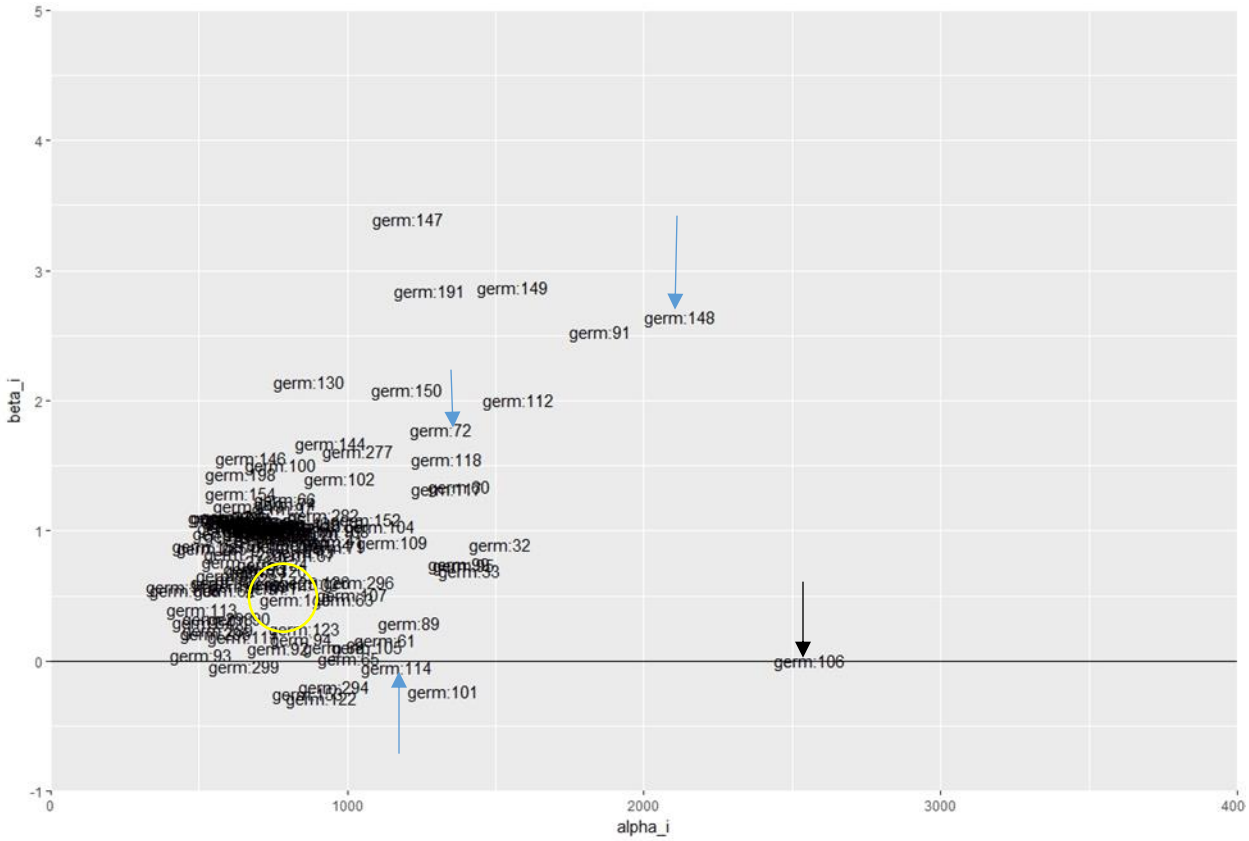


Figure 3- 6 Biplot with genotypic effect (αi) on the x-axis and environmental effect (βi) on the y-axis. The black arrow shows genotypes that have higher αi and lower βi. The blue arrow shows genotypes with high disease resistance and good yield. The yellow circle indicates the position of check cultivar Maui Lehua in the biplot.



APPENDICES

Appendix 1: Primers used for hi-plex genotyping.

Scaffold	Position	Forward Primer	Reverse Primer
74987	29	AGTGTCAGCCACAAC	CCAATATGCACACCACTGA
3738789	43	TGCTGCAGGGACAAATTC	TGTTTTAAATAAACCACTC
1793381	44	CATGCTCTGGCAGCAGACT	TTTGAATCTATTTGTTGGATTTTG
12124	56	GCCGCACCCCAGCATTTA	GCGGGGGTGTACAAAAAC
23348	66	GGATAAGGAAATGTGATCATTG	TTCACAAATCACTCTCGCAAA
869743	79	TTTCACTTTTATCAGAACTCAGAGG	TTTGCTTTAAACTGAAATACCC
1475552	80	CACTGAAGCAGAACTGAAACG	TTGATGACTTCACCTGGAAGG
3001389	84	GGACGAGTTCCCTCACGA	GAGGCTTTCTGCAGTCCAC
1893118	88	GCAATTGTAGCAGCAGCAAG	CCGCTGCGTCTTCACAC
4682330	92	CGCCGTCGTTCTCGTC	CGGGCTTTCACATGTATCCT
2309241	103	CACGAGCTCCTCCTCGTCT	CGTCGCCACGGATTTC
2541735	109	TTTCTGCAGATAAGGCTTGG	TGTCCACAATTTTGAGATGG
1478308	113	GAGGTTGTGGGGACCACTG	AGAGGGCATCCATCTCAGAC
3297248	113	CGTCCATGGGTGGAGAAG	ATATCACATATCTCTGCAGTATCG
2709283	116	TGTAGCGCTGCAGTGATTTT	TGACCCTACTAAATTGCCGTAA
134439	117	TTTGCACGTCAAATCCTTCA	TAGGTGCTGTCTTGGAGCAG
269380	119	GTGTACCCGGCTGCAGAAT	TACTTGGTGCCCCTACAAGC
2727943	129	CCAAGGACAACCGCTTATG	CCCCCAGAACTAGATGAGCA
171854	132	TGGATGGCGATGTAGTGAGA	TAACCACGCAGGGGTTCC
1394702	136	AGCTGCAGAAGACCCAAGAG	GTCCACTCTCTTCCCCGACT
6171750	136	TAGCTCATCGCCAAATCACA	TTTTTCGCCTTAACAAAGCTG
2388059	137	AGCCTCGGTCGTAAATTGAT	CGTGGCAAGGATGATAGAGC
470078	140	TTTCAGGCATCTTTCAAGGAG	CAGGGGACTTTGTTCACTATCC
6789385	143	GGGAGATGTGTACTGGCCTTT	TGCAGCAAGGGCAGATG
440289	144	CATTCTGCAGCCGTCCTC	AGTGGTGGAGGGTCTTGACA
170971	146	CCCTTTCTCTCCATCTTTTTCC	ACAGCCACTGTCTCCACAAG
722637	146	ACCTCAAGAGCTGCAGGAGT	CCCCAACCAGACTAGCAACA
325709	150	CTCCGCCGCGTTGGTC	GTTGCTTCAGACGGTGGAGT
2608330	150	CAGAAGCCAAGCGTCGAG	CAGTCCTCCCCAGACGTG
260119	151	ACCTCCCCTGTGCTTAGCTT	CTGGTGAAGCCCTTGGATAA
383107	157	AGCGTCGTCTGAGGAGAAAG	CTGAGGGCCATCTCCAAC
1033095	160	ACGGAGCAACCAAACAAGAG	CTTCGGTGAGGGGTTGTTAG
87402	162	GCCATATATTGCTTGTTCTTGC	GGTGGGCATGCTCAAAACTA
227030	162	ATGCTCTCGGCCAGCAC	GAGTTGAGGGCGAACTCGT
167481	164	CCAGCGTTGCCTCCAC	CACTAGGGACGCTGGTGGTA
1484055	182	CTAGACCTCCATGTGGCAAA	GCTATCCTGCAGAGGGAAGA
126223	195	ATTTCTGCAGGTCGCCATTA	TCCTCGAATTGAGCTGCTAAC
1172277	195	GGTTGGTTAACCTGTGAATGG	CCACTGGCTAAATGAGGTTGA

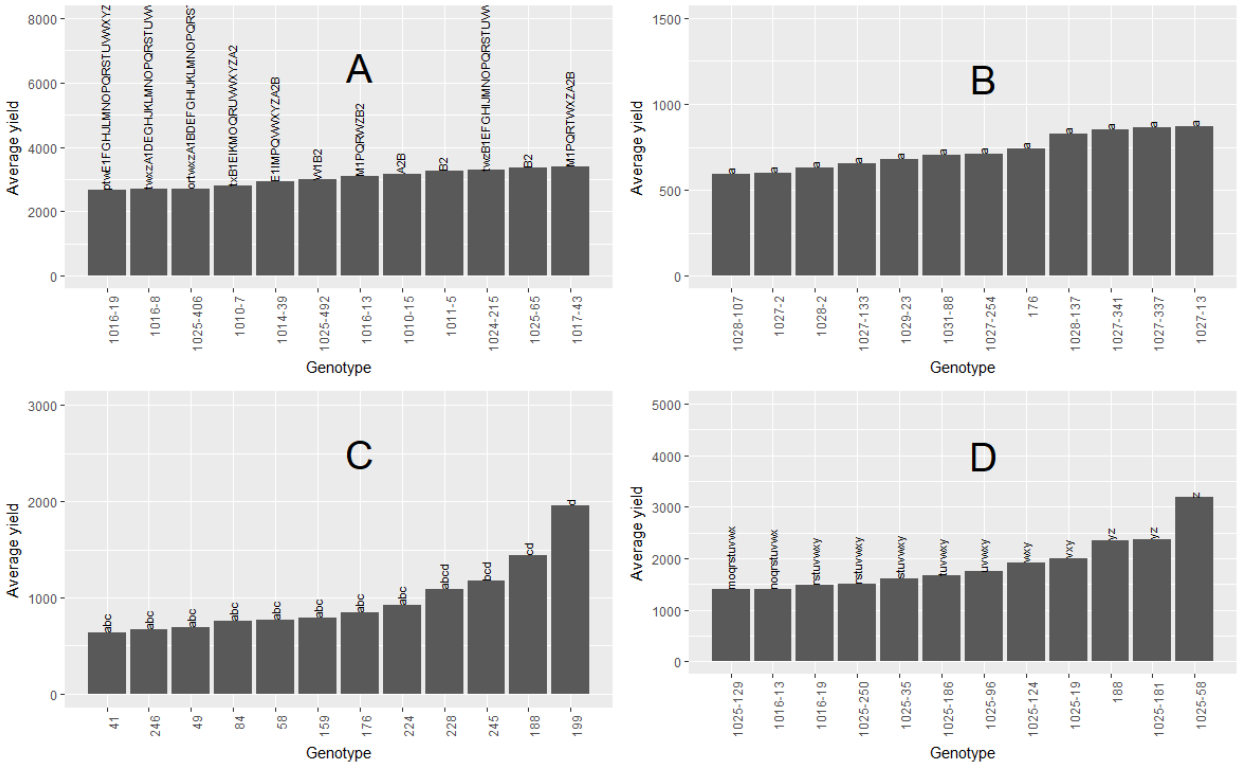
1647740	210	CGATCCAGTCGTACATGCTG	TCTGCTCTGCAGCCTCCT
813370	218	GAGGACAGCGACGTGGAC	GGGGCGGATAAGGTGTTG
167055	221	GCTTCTTCCTGAGGAGCTGA	GAGGAAGGATCGTGTTCCAA
2361750	229	AGCAGCCTGCAGAAGAGAGA	GCTGGAATGCTAGCTGTCTG
1014632	235	TTTTTCGGGTTCAACAGTGA	TCAGCTGCAGATGTGGTCAT
53202	237	AAGGTGCCATGAGTATGTTGA	AACCAGCTCTGAATCCATTT
196995	238	TCCAAACTCTAATGTTGCGATG	CCACACCTAGCTGCAGATTACA
41677	252	CTTTACTTTTCACTCAAATCTGC	AGTGCAATGGTGTGGATGAT
368549	262	CCACGACAAATAAATCCGACA	CGTGGCTCGTATCACTGTTG
1252928	266	CCTACAGGTCCCGAAGATGG	GGCGCTGTTTGCTCTCC
474221	279	CAAGAATATCACCAAACAGACTTG	CTCAACCTGCAGGATCCAAT
74730	295	GGAAACAGCATAATGAGAACATTG	GGCGAAGAAGTATGGTGCAG
1560849	303	CACTTGGTTGGTGTGGAGAA	CTGAACCCTGCAGCATCAT
608661	306	CCATACATCGATTGCTGTTTCA	TCGCTGTCACCGTCACC
238634	308	ATTTATAGAGCAGCGGGGAAG	GCCGGCCTGGCTACTT
1428525	317	CAGTGACCATTGTCCACTTCTT	AAAATCTGGAGGGACCTGTTC
1118010	343	CAAAGGTAAATGCAAATTCAGATG	CGCTGCAGGATAAATAAATGG
622526	360	GCAGTTTCAGGAGGTCCATC	TCTAACCTCGATTTGCAGTGG
128827	362	GGACGGTAAATTAAAGCTGGA	GGGTCTGTCTGCAAATCCTG
481335	370	TTCCCCTCAACCATTGCTAC	GCGATAGGTGACCAGGAAAG
73693	382	GTATGCCAGCATGCCTTCTC	GACCTGGACTGGGCTCTATG
810885	386	GCAGGTGCGCCCTCTT	CTTGGCGGCCACCAGAAC
233860	390	ACAAGGGGCCTGCAGAA	CCCCTTTTGCTCTCCTTTTT
123451	396	ACACTGCAGAGTTTGGAACG	TATCGAGGCCTTGGTCTTGT
484186	407	GAGGCCTCCGTATGAAGC	CTCCGGCAAAGCAAGC
390811	415	TTCTGAAGGTTTCATTGACTGC	CTGATGTCCAATTTGGCTTG
766973	423	CATAAGTGCCAGGTTCACCA	GGTGGGATCAGGGCTGT
91331	428	GAACCAGAGCCCCGTCTT	CTCAACGACCCGGACAAG
691575	431	ATTCTGCAGCCACCTTCATT	AGCAGTAAAGAACAGTGAAGAGA
739587	435	AGGCGGCAGATCAGGAATTA	GCACTCTTTTATCTCCTCCTCA
206265	448	CCAAAGTCCAGAAAATTGGAA	TCCCCGACACGAGAGTTTAT
88799	449	TGGCCAAACACAACCAGTTT	CCAGGATGGAACCTATCTGC
718775	459	CAGTTCATGGTTTGGCACTC	CTTCCCTCTTTTTCTCTGAACTC
92279	491	CCTGCAAGAACAATTTCCAA	GTGCGATCCATGGAGATACA
57883	494	CAAGGAAGGTGAGGAACCTG	GAGGCCGCCATGGAATA
96375	494	TGCATTAATGATTGTTCATCCTG	TGCTTCAAGTTTACTACATGCTCT
9713	496	GCTCGACAAAAAGGTTGCTC	CCTGGGAAGCTGTTGATCTG
68807	501	AGCAACACTTGCCCGATCT	GGCAGGCTTACTAGATTTTTGC
461636	530	ACGATTTCCCGCACAGTC	TATCGTCTCCGACACCTTTC
431519	532	GCCTCCTCCTTATGCAAGGT	GTTCGTGCATTGACGTTCAC
571916	537	GCAAGCAAATTGCCATGAT	CCTGCAGTCTGTTGGCAGAT
87806	542	GTTCTTTTCTGCAGCACGTC	ATCAAGGACAAGCACGAAGC
183564	549	GCCTCCACCTCCAAAGATG	GAAACGAACCCTCCAAACTG

30598	555	CAGCTGTATCTGCCCCTGTT	ACTGATGGTCCTGCTGGTTC
229673	556	TTGAGATTTGGGATGCCATT	CCTGCAGGATCACAATATCAGA
462117	562	CTGTCTCCCTGCGAGAGG	GCCAGCCATAGTAGGAGCAG
324831	583	CGGAGTTCTTGAAGCTGCTC	GGCGTTGGGGAAGGAC
484060	599	AGCATCAGCAGGGAGCTG	GGCATTGGCAGACATGG
178197	603	CAGTGGGGAGATCCAAAGG	CTGCATCACTTCCTCCACCT
57601	607	TCTTGTTGCTCCATAAACAGC	CCTCTAATCGGGGGTTGTTC
385297	693	CACGGCTGCAGCAAGTCT	GGTATGGCTCCAGGGACTG
75469	694	CAAATATTAGCAAAAATGTCTGTG	AGTATCATGCCTGCAGCAAA
398651	706	TGGAGCACAGGCAAGAAGAT	GCTGGGTTCCTTCACAAAAC
62735	716	GAGAATAGGAGCAACCTGCAA	CAGTCTTTGCGGTGCTGAG
99837	729	ATCTGCCTGTCGGTGCTC	CTTCGGGGTGCCGTTC
387327	749	CTGCGATGCCTACTATGAGC	GGTGTCTCCTGCTCTTCTGG
85388	754	TGTTTACAAGGACTCTTGGTTCC	TGTTTCTTCGCTTCATACCTG
319531	778	GACGACGGCATTCACTAAGA	CCGCAAAAATTCTGCAGGTA
97218	781	ACCGCGGCTTCCACCT	CATCCGCGTATACCTTGACC
326638	785	CCTGCAGGTTGATGAGGTACT	GCGAACACCTCCACCTTC
60309	791	CGGTGTTAAGGGACTTCTCC	TTGCAACGACATCACCTCTG
261459	798	ACCTGAAAGCGGGAGATTTT	CAGCCGCATGCACAGC
11419	806	CCTGCAGAAGTGGAGACAGA	CACCTGAACCTTCGGCTTG
174873	812	CACAAGGAATGGCTGGTGAG	AGGAACGGCGAACAAACAG
35477	820	GAACAGAAGTTGCCTACAAATCA	GGTGGGCAAAGAGTCTTGTT
50876	828	TCTGCAGGTTGGTGTGGTAG	CAGCTTAAAAATGATCATCTGACT
92688	846	TTTATTAAATTATCTGCAGTGGTG	CCATTAATACTTGCCTTCTTTGTG
237257	851	AATTTGGCCAACCTCATCAC	GCGGGATATAACCGTCGAG
29998	861	TGCCATGACTACCAAGCTGA	GATAACCTGGCTGGCACTTG
115080	864	TCGTCAATGCATTTGCTTCT	TCTGCGGAGCTAAATTCCTG
280862	865	AAGGGATGCCTCTGATGGA	ATGCAGAAGACATGGGATCG
8573	877	TAGGAGATGTTGCCCCTGAC	CACCAGCCTCGGCTACC
131392	892	GGTCACCTTGTTTCATGTGTG	AGTGGCAATATGGAGAGATG
6214	903	CCTGCAGGGGGCTTCC	GTCTGCAGGGAGGAGAGGTT
284185	911	AATATTGGATGGCACCCTTG	AGAAAAGCAACGTGCAGACA
36629	930	GAGGGCGGTGCTCTCG	ACCAGCCCAGGAACGAC
161603	938	GTGGCCTTGCAGTTCTTGAG	GAAGTCCTTCAACGTCACCA
190293	955	CCGCAGTACAGCAAATAGCA	GGGTTATCTGCAGCCATCAA
58971	960	TCATGGCTTCACGGCTTATT	AGAGCCTCCCCCAATCCT
136719	984	CGGATGCCTTAGAATGGAGTT	TCCTGCAGATCTAACCATGC
5143	989	GTTCCTGAAGGAGCTGATCG	CGCCCCATAGATAGAAGGAC
239881	999	AGCTGAAGATCTCCCCCTTC	CCCATCTTCCGTACACACG
150594	1015	CACCTCCTCTGCGAGTGGTA	CGGAAGAGGCAAAGTGGT
150969	1016	ATAATCTGCAGGCAATCACCA	ATTTTAACTCCCACACTTCTAGCC
230433	1017	TCCTTGGGTGAAGTAAAAGAATG	GCCCTCCTCCTTGTAGTGGT
90460	1061	TGAGACTGCTGCAGAATGGA	TAATGGCGGTAACCTTTGGA

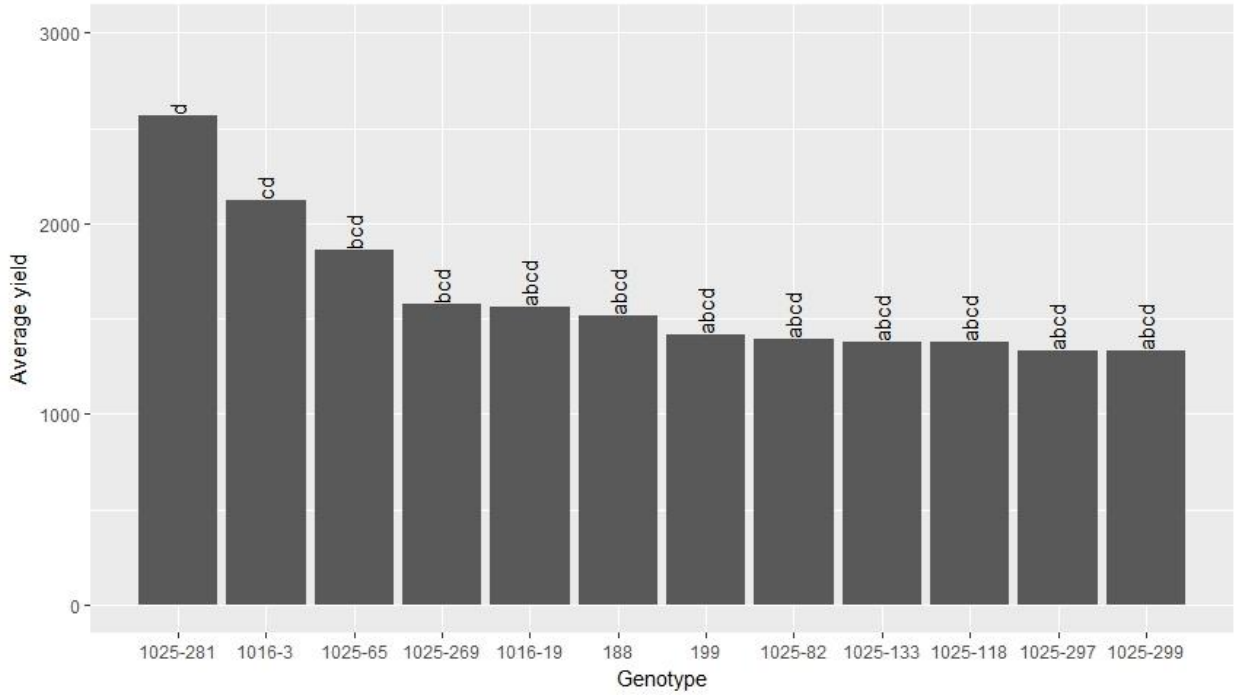
14899	1070	GCAAGGTGAGAAATTGCTATTGTA	GGCCACCTCACGAACTATCT
175544	1128	CCTATCCCTTCCTTGGGATT	ATGCTTCTGGAGAGGTGCTG
105190	1130	TCATTAGCCTTGCCATTGAG	CTGATGATGTGCCATAACTGC
2165	1166	ACATCTGGGAATCACCCAAG	AGAAGACCTCGTGGGCAGA
81930	1258	TGAACCTGCAGAGCTCTCACTA	CTGAAGTGCATCGGACCTCT
176686	1260	TGACCCTTGTGTGGTTTGTC	TGACAAGGACCTGAGAGGCTA
18546	1270	GGGGTCCCAGCAGGAG	TGCTACACCCCCACTAGTCC
25328	1294	GCAGAAACTGCTGGATGACC	GTCGCTTGACCTGAGCACAT
114063	1298	ACATACATATGTGGTGTTGTGC	AATAAATCAGGACTCCCAATGC
52376	1335	CGTCGTAAGGAGGAAACCAG	ACATACCCTGCAGCACCAGT
63297	1335	TTCCTAAGACTGTCATCCAAAATC	CATTCTTTTCCCCCACCA
102848	1346	GGTAAGGGCCATGAAGAGG	ACCAGCAGGAAAAAGTCTGC
118724	1350	GACTAGTTGGGGCCAGCA	TCGGTGCTGGACTACTGC
34599	1381	CTTTCTGTACCGGGTCATGG	TTCATCTAACTGGCGTTTGG
8208	1390	AACACCACCATTAGAATTAGCA	CCTGCAGCTTCTTCCTTCTG
100620	1391	TCAGTCCTCCATTGACCATGT	TGCAGCTGTAGTGATGGAGA
12857	1400	CATATCGTGGCGAATCAGAA	CAAGGTTCGCCGCATC
13527	1401	CGATCCATCTTGGAAGCTACA	GCAATGGACACATTGACTATCTC
27116	1401	ACTGCAGCTTTGGATGGACT	ATTTCCTGTGAGGGGTTCTG
27772	1452	CAACGAGTCACCTCCTTGGT	CAACACCTGCCATTGATGAT
47202	1495	AGGCTTGCAGCCCTCTG	CAGCTCGTTGGGTTCCAG
24549	1509	TGGCAGAGAGCAGTCACTTG	TGGATTGAAAATTCTACTGATTC
72382	1521	CATGGACGTCGGTGGTG	GAGTCGTCCGCCAGGTG
70603	1547	GTGGGGACGTTGCTGTG	GGCATGGCCTATCTCCAATA
107888	1588	CAAGTGACTGCAGTGCCTACT	AATCCCCAGCCATCCATT
2921	1600	CCGTTTAATTACACACGCTTCA	TCATCGATCCCCATATGTCC
88366	1667	CATGGATCTGGTCTGATGCT	GCAGTAAGGCAATATCCAAAGG
80922	1692	TTCACCCTGCAGAAGGACTC	TGCAGAGAGGGAGCAACC
29097	1737	CCTACTGGGTCTGGTTCCAC	GGGAACCTAAGCTTCTCTACTCG
31698	1791	GCAAGTTTCGCAGCATTTTG	GATCGGCATCGGGTTTCT
12826	1792	CAGGCACCCCATCCTG	GGAAGTTGTCAGGAGGAACC
10223	1799	CGATGAGGCCGAGAGAAATA	CACGCTACATATGCAGGTCCTA
33542	1880	TGCAGTTACTTGGCTCTATCA	TGGCATTCTCACTTCCTCTCT
35184	2009	AATCTCTTGCGCGTTTTGAG	GCCTCAAGTCGCTACGATTC
14631	2057	ATCCAGTCCGGCTGCTG	CGAGATTGCTAGGAAGACAGG
15255	2074	ACCAGGTGGAAGATAATTCAGC	AGACTGCAGTAATTGGATGGTC
14478	2077	TGGGATTTGACAGTTTCCTTG	TGGGTGGCACTATGCTCTAA
69776	2168	GCTGTGCTCTCTCGCCTATC	AGAGAGGTGGTGGGAGATGA
18948	2301	TTGCTACGGTTCTGTTTTGC	CCACCAGCTCTTCTTCAACC
26662	2363	GGAGTCTCTCCTGTTCCTTGC	GGGACGAGATGTTGAAGCAG
11974	2404	ATCACAGCAAGAGGTGACCA	CCACGTTACTGATGCTCTGC
6294	2452	CGACCATGCAGGCGTACT	CTTCGAGCAGATGGAAGAGC
34999	2460	ATCAGAATGTCCGCCAGAGT	GGACATGGGAGTAGAACAGCA

33703	2756	TCTGCGTCATCCTCCTCTG	AGGCCCCGGACGACAG
17876	2885	CATGGATATCTTGGACACAGGA	ACTAGGCTGCAGGTGTTTGC
16931	2930	CAGGGAGTTTTGGTTCTCTCA	AGAAGCCTCCACCCTTCTCT
1375	3008	AGCTGAGCAGTAGTAAGGAGGA	TGCAGCTTGAGTTTACTCTTCA
2570	3015	TTGGTCGTCCAGGGAGATAC	GCAGGTGACAGGCATCC
832	3235	ACATGTACTGGGCCAATTTT	ATGTTTTGAAACAGCCTTACCA
4254	3420	GCAGAAGCTCCGGAAGG	GGGGTTCTGCTCCCTGTAG
5983	3608	TGTTTGACAGCCATGAATCC	GCCCAGCCCATGTAAAATAA
9098	3929	TCAAACCCCACTGTTAAAAAGA	GCAGCATTGGACCTTTTGTT
6966	3959	CGAGCAAAGCTCTTAGTGGTG	TCAGCTAGCTAGTGGGGACTG
2823	4049	TCTGTACCAGCAGAGAGCATGT	TCCCATCTGATACAGAACTCCA
3612	4328	GCCATGCACCCATCGT	CCGCGGCATCAACTCT
6403	4791	TTGTGTTTGATCTTTCCTTGG	CTTTCAGTTCTCGCCAGATG
2708	5341	ATGGGGGCCACAGCTT	CGTCCGCATGAGTTTGC
5107	5487	CAATGCCCCAAGAGGAAGT	CCACCACCAGCAGTGGA
370	8364	GGTTGTCAAAGGGGAGTTTG	CACCTCCAGAGCTTCCATTA
322251	344-347	TGCAGCATTAGGAGGATGC	TGCAGAAGTGGGAGGAAAAT
2112265	98-108	CAGGTTCGCCCGCAAG	AGACTTTGTCTTGCAG

Appendix 2: Mean comparison of the genotypes using model 1 for the year A) 2013, B) 2014, C) 2017, and D) 2018. Model 1 explores the within year variation between genotypes. Bonferroni correction at alpha = 0.5 was used. Genotypes with different letters are significantly different. No significant difference in yield was found between the genotypes for these four years. Only 12 genotypes per year are shown in the bar plot.



Appendix 3: Mean comparison of genotypes using model 2 for six years. Bonferroni correction at alpha = 0.5 was used. Genotypes with different letters are significantly different. Only 12 genotypes are shown.



Appendix 4: Publication list.

Peer Reviewed Papers

Martin Helmkampf, Thomas K. Wolfgruber, M. Renee Bellinger, **Roshan Paudel**, Michael B. Kantar, Susan C. Miyasaka, Heather Kimball, Anne Veillet, Andrew Read and Michael Shintaku. 2017. Phylogenetic relationships, breeding implications, and cultivation history of Hawaiian taro (*Colocasia esculenta*) through genome-wide SNP genotyping (Journal of Heredity)

Non-peer reviewed papers

Miyasaka SC, Bellinger MR, Kantar MB, Helmkampf M, Wolfgruber T, **Paudel R**, Shintaku M (2017) *Genetic diversity of Taro*, p. __. Nandwani D (ed) Genetic Diversity in Horticultural Plants, Springer-Verlag, Switzerland. **(accepted**)

Professional Presentations

2017. CTAHR/COE Student Research Symposium, Genetic Improvement of Taro for Taro Leaf Blight resistance.

2017. ASHS Annual Conference, Genetic Improvement of Taro for Taro Leaf Blight resistance in Hawai'i, Waikoloa, HI.

2018. Northern Organic Vegetable Improvement Collaborative (NOVIC) organic breeding workshop, Introduction to taro and tropical perennials breeding program.

2018. CTAHR/COE Student Research Symposium, QTL mapping of resistance to Taro Leaf Blight disease in taro (CTAHR MS Student Poster Presentation Award of Merit).

2018. CTAHR/COE Student Research Symposium, Three Minute Elevator Pitch (3MEP) competition.